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Exploring soybean integrated pest management in a changing agricultural environment: the impacts of decreasing ecosystem services, invasive species and specialty cultivars

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**Exploring soybean integrated pest management in a changing
agricultural environment: the impacts of decreasing ecosystem
services, invasive species and specialty cultivars**

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Entomology

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Abstract

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is a recently invasive pest to North America. Integrated pest management strategies exist for minimizing the impact of the soybean aphid on yield. These strategies were developed from field-based research conducted under the environmental conditions of the time and using commodity soybean plants. The increased use of broad spectrum insecticides and the increased simplification of the landscape along with the release of altered fatty acid soybean cultivars have led to changes in the agricultural environment. How these changes affect soybean aphid population dynamics and interactions between the soybean aphid and other pests is investigated. The first objective was to determine how reduced natural enemy services may impact soybean aphid population growth between the economic threshold and economic injury level. The second objective was to determine how the soybean aphid could indirectly interact with the soybean cyst nematode, *Heterodera glycines* Ichinohoe and the brown stem rot fungus, *Cadophora gregata* Harrington and McNew. The third and fourth objectives were to determine the effect of altered fatty acid cultivars on soybean pests and pathogens. Objective four quantified the effect of these cultivars on the performance of the soybean aphid, soybean cyst nematode and brown stem rot. Objective five addressed how the alterations in fatty acid synthesis pathways present in these cultivars may affect plant volatile emissions and the downstream impact this has on host plant selection by the bean leaf beetle, *Cerotoma trifurcata* Forster (Coleoptera: Chrysomelidae).

Chapter 1

General Introduction and Literature Review

Thesis Organization

The research covered in this thesis seeks to refine current knowledge on the application of integrated pest management for Iowa soybean production. The thesis is organized into six chapters. Chapter one contains a literature review and an introduction to the research presented in later chapters. Chapter two will report on the functionality of the current economic threshold for soybean aphids in a natural enemy free environment. Chapter three will detail the interaction of soybean pests and pathogens across conventional and modified fatty-acid cultivars. Chapter four will explore the efficacy of current integrated pest management recommendations for modified fatty acid cultivar production. The response of bean leaf beetles to plant volatiles from soybean cultivars will be reported in chapter five. Finally, chapter six will provide a brief summary of the conclusions from the research reported in this thesis.

Introduction and Literature Review

Low Linolenic acid cultivars of soybean, *Glycine max*

Oil derived from the seeds of *Glycine max* Merr. (soybean) accounts for 80% of all vegetable oil consumed in the United States (DiRienzo et al. 2006). The seed produced from commodity soybean contains linolenic acid levels of around 7% (70-

100 g/kg) (Chappel and Bilyeu 2006). The oil produced from these soybean seeds is oxidatively unstable, due primarily to the three double bonds in linolenate.

Manufacturers hydrogenate the oil to stabilize it. Hydrogenization is a process in which hydrogen atoms are linked to the carbon atoms of fatty acids. This process increases the shelf life of the oil and also gives it a more desirable texture. The process of hydrogenization leads to the production of saturated fats and trans fats (Chappel and Bilyeu 2007). As of January 2006, the Food and Drug Administration requires all food manufacturers to provide trans fatty acid content information on labels. This regulation came as a response to research that trans fatty acids have negative effects on blood cholesterol levels, leading to increased risks for coronary heart disease (Department of HHS and USDA 2005).

Currently specialty cultivars with altered linolenic acid contents have been developed with linolenic acid contents around 3%. Specialty ultra-low linolenic acid cultivars have also been developed with linolenic acid contents around 1%. These specialty low-linolenic acid and ultra low-linolenic acid cultivars have low enough alpha-linolenic acid contents that the need for chemical hydrogenization is partially or entirely eliminated, leading to lower saturated and trans fatty acid contents. These specialty cultivars have greater commercial value due to the improved oxidative stability of the oil produced from the plant and the potential health benefits that they offer (Ross et al. 2000).

The genetic changes present in low linolenic cultivars have been developed through the conventional breeding method of chemical mutagenesis. Chemical

mutagenesis was achieved by treating seeds with ethyl methanesulfonate. Other lines of low linolenic cultivars were developed by X-ray irradiation of seeds (Rahman et al. 1996). These mutagenesis procedures caused alterations at the *Fan* locus. These mutations affect the microsomal omega-3 fatty acid desaturase (*FAD3*) genes, which codes for enzymes. The *FAD3* enzymes are responsible for introducing the third double bond into linoleic acid precursors to create linolenic acid precursors (Bilyeu et al. 2005). The *FAD3* enzymes are part of the microsomal fatty acid biosynthesis pathway that is important in producing fatty acids for storage oil in developing seeds (Anai et al. 2005). There are three genes that code for *FAD3* (*FAD3A*, *FAD3B*, and *FAD3C*) in the soybean genome (Bilyeu et al. 2006). Low linolenic acid cultivars contain either deletions or modifications of one or more of the *FAD3* genes (Fehr 2007). These mutations act in an additive manner to lower linolenic acid content (Rahman et al. 1994.)

Linolenic acid in *Glycine max*

Linolenic acid is one of five major fatty acids present in soybean (Fehr, 2007). These lipids are involved in several important processes in plants including, providing a physical barrier on epidermal cell surfaces, and chemical signaling of stress responses (Shah 2005). In the plant's defense against pathogens and herbivores linolenic (18:3) and linoleic (18:2) acid are the two most common substrates of lipoxygenases. Lipoxygenases produce important oxylipins in the

plant's response to herbivory and pathogens. Oxylipins are a group of compounds, which are produced by fatty acid oxidation. They serve various functions in the cell (Porta and Rocha-Sosa 2002). These important oxylipins include jasmonic acid (JA) (Kaloshian and Walling 2005).

The lipoxygenase pathway also produces the six-carbon green leaf volatiles. These volatiles along with terpenoid and phenylpropanoid volatiles are released from the site of tissue damage upon wounding (Bate and Rothstein 1998). The volatiles produced are signal molecules capable of alerting the plant upon tissue damage. In the case of tissue damage due to herbivores they are also capable of providing direct defense through deterring further herbivory and also providing indirect defenses through attracting natural enemies of the herbivores feeding on the plant (Baldwin et al. 2002, Choudhary et al. 2008, Halitschke et al. 2008).

Green leaf volatiles produced by linolenic acid accumulate in response to aphid feeding. They act directly to repel aphids and attract predatory insects. Green leaf volatiles will also prime a plant to allow elevated levels of JA when herbivore oral secretions are present (Kaloshian and Walling 2005 and Walling 2000).

The plant hormones salicylic acid (SA) and Ethylene (ET) along with JA are three signaling molecules known to play key roles in amplifying initial plant response to both biotic and abiotic stresses. These three compounds function together through a complex set of regulatory interactions (Shah 2005, Smith and Boyko 2006 and Krunkel and Brooks 2002). They influence one another through both positive and negative interactions. The primary modes of interactions include, positive

interactions between JA and ET and negative interactions between SA and JA, and SA and ET (Rojo et. al 2003).

Plant response pathways to stress can be divided into two categories SA dependent and JA/ET dependent. The SA dependant pathway is known to play a role in defense against biotrophic pathogens and some insect herbivores including phloem-feeding insects. The pathway is characterized by a hypersensitivity response (HR). The HR is a cell death process induced by a pathogen. The HR response is dependent upon nitric oxide and reactive oxide species. These molecules increase in the plant after pathogen attack and promote the HR response and stimulate SA production (Walling 2000 and Shah 2005). Lipoygenases are most likely involved in the loss of membrane integrity in the HR (Porta and Rocha-Sosa 2002). The SA pathway also promotes the development of systemic acquired resistance (SAR). SAR develops following initial infection by a pathogen that causes tissue damage at the site of infection. SAR provides a state of heightened resistance throughout the plant (Kunkel and Brooks 2002).

The second category, the JA/ET dependent pathways can be divided into two separate pathways, the wound response pathway and the induced systemic resistance pathway (ISR) (Walling 2000). The wound response pathway is triggered by herbivore feeding and wounding. Chewing, phloem feeding, and cell content feeding herbivores along with necrotrophic pathogens activate the wound response pathway. The pathway results in the production of proteinase inhibitor

(PIN) genes. PINs interfere with digestion in the insect gut and deter further feeding (Maleck and Dietrich 1999).

The other JA/ET dependent pathway is characterized by an ISR. An ISR can promote resistance to certain pathogens and is triggered by the colonization of roots by certain non-pathogenic bacteria. The resistance is characterized by a heightened state of alert in which the plant responds more rapidly and strongly to challenge by pathogens (Shah 2005 and Rojo et al. 2003). It is not known whether any herbivores activate an ISR (Walling 2000).

***Glycine max* pathogen and pest complex**

Glycine max production in the United States faces multiple challenges from pathogens and pests. Viruses, fungi, nematodes, chewing insects, and piercing/sucking insects have all been identified to afflict soybean. In this thesis four pathogens/pests were chosen to assess their ability to afflict low linolenic acid specialty cultivars. The organisms *Heterodera glycines* (soybean cyst nematode), *Cadophora gregata* (brown stem rot), *Aphis glycines* (soybean aphid) and *Cerotoma trifurcata* (bean leaf beetle) were chosen based on their representation of four different pathogen/pest groups; nematodes, fungi, piercing/sucking insects, and chewing insects respectively. These pathogens/pests were also chosen for the region of the plant that they afflict. *Heterodera glycines* infects the roots (Niblack et al. 2006), while *C. gregata* infects the plant systemically (Allington and Chamberlain

1948), *A. glycines* infests the outer leaf and stem tissue and phloem of the plant (Walter and DiFonzo 2007) and *C. trifurcata* feeds on the leaves, stems and pods (Smelser and Pedigo 1992b). These four pathogens/pests were also selected for their economic importance as yield reducing factors in Iowa soybean production.

***Heterodera glycines*, soybean cyst nematode biology and ecology**

Heterodera glycines Ichinohe, the soybean cyst nematode, was first discovered in the United States in 1954. It is now present throughout all major soybean-producing states in the U.S. (Schmitt et al. 2004). *Heterodera glycines* is considered to be the most economically important pathogen of soybean in the United States, where it is estimated to cause over \$1.5 billion a year in damage, and is capable of reducing yields by up to 50% (Niblack et al. 2006).

Heterodera glycines is a sexually dimorphic obligatory endoparasitic roundworm that infects the roots of soybean plants. The lifecycle of *H. glycines* can be divided into five stages, four juvenile stages and the adult stage. The transition from the first juvenile stage (J1) to the second stage juvenile (J2) occurs within the egg. The J2 stage emerges from the egg and infects plants by entering the roots and establishing a specialized feeding cell termed a syncytium. The syncytium is established within or near the vascular tissue. The third (J3) and fourth stages develop within the roots. Sexual dimorphism first appears in the J3 stage as females begin to swell, and become lemon shaped. At this point the females become too large to be contained inside the root and are visible on the outside of the roots of

soybean. Although adults of both sexes swell and lose their motility, males will regain their motility after inseminating females. The males will then exit the roots. The females however, remain attached to the plant root. Each female is capable of producing up to 600 eggs, of these as many as 200 will be secreted in a gelatinous mixture. The eggs contained in the gelatinous mixture are more likely to hatch during the same season. The rest of the eggs will remain inside the female in the cyst formed by her body. The eggs inside a cyst can remain viable for as long as 11 years (Niblack et. al 2006 and Tylka 1995).

Heterodera glycines has a wide genetic diversity between and within populations that allows it to readily adapt to resistant soybean cultivars (Niblack et al. 2002). This genetic diversity coupled with its ability to sustain populations within a cyst for up to a decade, that are large enough to cause yield loss, requires a long-term management program. Hundreds of *H. glycines* resistant soybean cultivars have been developed to limit the damage caused by the pest. These cultivars incorporate resistance from three soybean source lines of resistance, PI 88788, PI 209332 and Peking. Of the numerous *H. glycines* resistant cultivars available over 95% contain PI 88788 derived resistance (Tylka et al. 2010). Current management relies on a six-year crop rotation scheme that includes crop rotation between non-host plants (i.e. corn, oats, and alfalfa), resistant soybean cultivars containing different sources of *H. glycines* resistance, and susceptible soybean cultivars (Niblack et al. 2006).

***Cadophora gregata*, brown stem rot biology and ecology**

Brown stem rot which affects soybean, is caused by the fungus *Cadophora gregata* Harrington & McNew (Harrington and McNew 2003) (*Phialophora gregata*). *Cadophora gregata* was first discovered in 1944 in central Illinois (Allington and Chamberlain 1946.) *Cadophora gregata* is an important pathogen that occurs frequently in the north central United States, where it can cause yield losses between 12 and 38% (Waller et al. 1992). It is prevalent in 68-73 percent of fields in Illinois, Iowa, and Minnesota (Workneh 1999).

Cadophora gregata is a spore forming fungus that overwinters saprophytically in soybean debris that was previously parasitically infected. *Cadophora gregata* only reproduces asexually and does not form any long-term survival structures. The fungus initially infects the soybean plant through its root system. Once pod formation begins the fungus will spread to the stems via the vascular tissue. There the fungus causes a progressive browning of the vascular and pith tissues, which impedes the movement of nutrients (Allington and Chamberlain 1948). Two genotypes exist of the *C. gregata* fungus, genotypes A and B. The two genotypes differ in their ability to cause foliar symptoms on susceptible soybean. Genotype A is considered to be the more aggressive type, as it can cause interveinal chlorosis and necrosis. This leads to the curling and wilting of the leaves. Genotype B does not cause any foliar symptoms (Tabor et. al 2007).

Soybean is the only host of *C. gregata*. *Cadophora gregata* also lacks a long-term survival structure. These two factors contribute to the current management strategy of crop rotation, tillage and the use of resistant cultivars (Adee et al. 1994).

***Aphis glycines*, soybean aphid biology and ecology**

Aphis glycines Matsumura is an introduced pest of soybean in North America. *Aphis glycines* was first reported in Wisconsin in 2000 (Ragsdale et al. 2004). Since its introduction into North America it has become the leading insect threat to the agricultural production of soybean in the United States (Ragsdale et al. 2007). Yield losses of between 15 and 40 percent have been recorded due to *A. glycines* herbivory. In 2003, 57.7 million bushels in yield loss due to *A. glycines* was reported in Iowa (Rice et al. 2007).

Aphis glycines has a heterecious holocyclic life cycle. Eggs hatch on *Rhamnus* spp. (Rhamnaceae) in the spring. A fundatrix emerges from each egg and begins to reproduce asexually, giving rise to apterous viviparous females. Asexual reproduction will last for three to four generations. Alate viviparous females (winged adult females) will begin to appear during the third and fourth generation. The alate viviparous females will leave the *Rhamnus* spp. to find their secondary host, soybean. On soybean, *A. glycines* will reproduce asexually with populations capable of doubling every 1.5 days. Later in the growing season, plant senescence and photoperiod reduction stimulate gynopara production. At this time, winged males are also produced. The gynopara migrate back to *Rhamnus* spp. where they

produce ovipara that mate with winged males. The eggs produced are most commonly deposited at the base of buds of *Rhamnus* spp. (Ragsdale et al. 2004).

Aphis glycines causes damage to soybean by feeding on the phloem of the plant, removing photo assimilates resulting in decreased seed weight, number of seeds per pod, and pod number. All of these factors lead to decreased yield (Ragsdale et al. 2007). *Aphis glycines* can also cause damage to soybean indirectly by vectoring viruses (Hill et al. 2001 and Clark and Perry 2002). Current management practices for *A. glycines* include seed treatments and foliar applications of insecticides. Foliar insecticides applied based on growth stage of the plant (R3-R4) or according to an established economic threshold (250 aphids/plant) have been shown to provide consistent and effective yield protection (Myers et al. 2005, Johnson et al. 2009). Recently, host plant resistance (HPR) genes against *A. glycines* have been identified (Hill et al. 2006, Mian et al. 2008, Zhang et al. 2009, Zhang et al. 2010). The first of which has been incorporated into commercial cultivars (Kim et al. 2009, Mardorf et al. 2009). Biotypes of *A. glycines* capable of overcoming these HPR genes have been identified (Kim et al. 2008, Hill et al. 2010). It is still not known if in the field HPR genes alone are capable of maintaining *A. glycines* populations at or below the economic injury level.

***Cerotoma trifurcata*, bean leaf beetle biology and ecology**

Cerotoma trifurcata Forster is native to the United States and in the North Central United States it is a sporadic pest of soybean. *Cerotoma trifurcata* is capable

of reducing soybean yield through early season transmission of bean pod mottle virus (BPMV) and late season pod injury. Pod injury allows for secondary fungal infection to reduce grain quality (Paul 1989).

In Iowa, *C. trifurcata* has a bivoltine life cycle. *Ceratomyza trifurcata* overwinter as adults (F_0), primarily in wood lots (80%) and soybean fields (20%) (Lam et al. 2002). In early spring *C. trifurcata* adults emerge from their overwintering habitats to feed on naturally occurring legumes and alfalfa (Smelser and Pedigo 1991). Adults will move into soybean fields at plant emergence and begin feeding, ovipositing and transmitting virus if infected. The F_1 generation that emerges in soybean in mid- to late June is a secondary source and the primary dispersal agent for BPMV. The F_2 generation is not likely to be an important vector for BPMV (Giesler et al. 2002), but instead is capable of causing damage to pods thereby reducing yield and grain quality (Smelser and Pedigo 1992a).

Management of *C. trifurcata* in Iowa relies primarily on the use of insecticides. The overwintering and F_1 generations are targeted with either a seed treatment or a foliar application of insecticides. These two management practices have been shown to be capable of reducing late season populations of *C. trifurcata* and decreasing the incidence of BPMV (Krell et al. 2004, Bradshaw et al. 2008). Delayed planting date has also been shown to decrease late season *C. trifurcata* densities (Pedigo and Zeiss 1996), but this management technique has inconsistent impacts on BPMV incidence (Krell et al. 2005).

***Glycine max* pathogen and pest indirect interactions**

Aphis glycines and *Heterodera glycines*:

The effects of *H. glycines* infections on *A. glycines* populations is not well understood. Preliminary investigations were attempted jointly by researchers at Iowa State University and the University of Illinois at Urbana-Champaign (F. Avendano pers. comm.). The two pests were initially hypothesized to be competing for resources as both pests feed at or near the vascular tissues of the plant. Initial field data, however, suggests that the two pests do not affect each other. Data have suggested the two pests have an additive effect on decreasing plant height and yield. In field micro-plot studies the joint infestations of *H. glycines* and *A. glycines* resulted in a greater yield loss than by either infestation alone. Seed quality does not seem to be significantly affected by a dual infestation of *H. glycines* and *A. glycines* (F. Avendano pers. comm.).

More recent research conducted by Hong et al. (2010) investigated the influence of *H. glycines* infection on *A. glycines* preference and performance. In a controlled greenhouse environment, *A. glycines* alates were found to significantly prefer uninfected soybean plants to those infected with *H. glycines*. In this study, results for *A. glycines* performance were inconsistent with neutral or slightly positive impacts of *H. glycines* infection on *A. glycines* performance. In a follow-up field experiment *A. glycines* preference and performance was measured across varying densities of natural *H. glycines* infestations. In the field *A. glycines* preference was also influenced by *H. glycines* infection (Hong: unpublished data).

Performance of *A. glycines* however, was unaffected by *H. glycines* infection in the field.

Cadophora gregata and *Heterodera glycines*:

Increasing populations of *H. glycines* have been shown to increase both the incidence and severity of both genotypes A and B of *C. gregata* in soybean (Tabor et. al 2006). Increasing populations of *H. glycines* also causes earlier colonization of soybean by *C. gregata*, which could lead to greater yield losses. This increase in severity occurs in both *C. gregata* susceptible and resistant cultivars (Tabor et. al 2003, 2006). It also occurs in both *H. glycines* susceptible and resistant cultivars (Tabor et. al 2006). Due to the yield loss potential of the combined infections of *H. glycines* and *C. gregata*, current management strategy is to use cultivars with resistance to both pathogens. The use of such cultivars was shown to be successful in reducing the affects of *H. glycines* on *C. gregata* infections.

The mechanism by which *H. glycines* affect infection of soybean by *C. gregata* is not yet understood. It has been hypothesized that physical wounds in the roots caused by *H. glycines* feeding, movement, growth and reproduction may provide a direct route of entry for *C. gregata*. These wounds might also adversely affect the plant defense in the surrounding tissues. Lastly, it is also possible that *H. glycines* infection may compromise the plant's general health and reduce its resistance to infections (Tabor et. al 2003).

Aphis glycines and *Cadophora gregata*:

The possible effects of *C. gregata* infections on *A. glycines* populations have not previously been studied. It has been shown that the chemical composition of a plant can be modified by the metabolic activity of the fungus and plant following initial fungal infection. These chemical changes can be due to pathogenesis-related enzymes produced by fungal infections. These enzymes are capable of spreading systemically throughout the leaves of the plant (Moran 1998).

Phenolics are another plant defense chemical response to fungal infection. Phenolics are formed in response to the invasion of a pathogen. They accumulate rapidly at the site of infection and work to slow pathogen growth (Nicholson and Hammerschmidt 1992). Phenolics have been shown to be capable of altering insect feeding, survival, and reproduction. Phenolics, however, occur in small levels in phloem sap. Studies have also shown that aphid performance was unaffected by elevated phenolic compounds in silver birch leaves (Johnson et. al 2003). The role phenolics play in soybean response to *C. gregata* infections, and its impact on *A. glycines* is unknown.

The indirect interactions of plant fungi and aphids through chemical changes created by fungal infections in the plant have been studied in other systems including *Cucumis sativus* L. (cucumber), *Cirsium arvense* L. (creeping thistle) and *Betula pendula* Roth (silver birch). In creeping thistle infected with *Puccinia punctiformis* (Str.) Rohl (rust fungus) and silver birch infected with *Marssonina betulae* (Lib.) Magnus, aphids were more abundant on leaves infected with fungi

than on uninfected leaves (Kluth et. al 2001 and Johnson et. al 2003). On silver birch, aphids were found to also reproduce better on infected leaves than uninfected leaves (Johnson et. al 2003). In cucumber plants infected with *Cladosporium cucumerinum* (the cause of cucurbit foliar necrotic scab), aphids had increased production of nymphs on locally infected leaves, while aphids on systemically infected leaves showed no difference in reproduction when compared to aphids on uninfected leaves (Moran 1998).

The response of many plants to fungal infection is similar to physiological changes that occur during senescence (Johnson et. al 2003). This response includes an increase in free amino acids and nitrogen in the phloem (Kluth et. al 2001, Thomas and Stoddart 1980). Obtaining nitrogen is the primary nutritional challenge facing aphids. Amino acids in the phloem are thought to be the only source of dietary nitrogen for aphids (Walter and DiFonzo 2007). Increased levels of free nitrogen in the phloem of soybean have been shown to cause increased aphid population growth (Myers and Gratton 2006). The infected cucumber, silver birch, and creeping thistle plant systems (Table 1) are consistent with uninfected plant systems in which aphids show preference for senescing leaves. The similar reproductive ability of aphids on uninfected and systemically infected leaves of cucumber may be the result of metabolic sinks. Metabolic sinks occur when infected tissues, in the same manner as young plant tissues, draw, accumulate, and use compounds at the cost of older tissues (Barbosa 1991).

The indirect interaction of *A. glycines* and *C. gregata* on soybean is currently unknown. Research conducted on the cucumber, creeping thistle, and silver birch systems suggest that the interaction could lead to an increase in host suitability for *A. glycines* due to an increase in free nutrients in infected soybean.

Objectives and Hypotheses

Chapter Two

Contribution: Collection and analysis of all data presented

- Assess the impact of a predator-free environment on soybean and *A. glycines* populations.
 - I hypothesize that in the absence of predators the current economic threshold (ET) will still protect yield loss from *A. glycines*
 - I hypothesize that in a predator-free environment *A. glycines* will increase in density from the ET to the economic injury level in less than the seven days recommended by Ragsdale et al. (2007).

Chapter Three

Contribution: Collection and analysis of all data presented

- Assess the impact of low linolenic acid cultivars on soybean pest and pathogen performance.

- I hypothesize that *A. glycines*, *H. glycines*, and *C. gregata* will have improved performance on low linolenic acid cultivars when compared to commodity cultivars.
- Assess the ability for soybean pests and pathogens to interact indirectly through a shared host plant.
 - I hypothesize that the performance of *A. glycines*, *H. glycines*, and *C. gregata* will be reduced when all three organisms share a single plant.

Chapter Four

Contribution: Collection of yield data, analysis of yield and linolenic acid data

- Assess impact of soybean pests on altered linolenic acid soybean cultivars.
 - I hypothesize that current integrated pest management recommendations will also provide yield protection for low linolenic acid cultivars.
 - I hypothesize that pest populations left unmanaged will elevate the seed linolenic acid content of low linolenic acid cultivars.

Chapter Five

Contribution: Collection and analysis of all data presented

- Assess impact of prior *C. trifurcata* on beetle preference for soybean plant volatiles.

- I hypothesize *C. trifurcata* will be preferentially attracted to the volatiles of plants with prior feeding damage from *C. trifurcata* compared to intact soybean plants.
- Assess impact of altered fatty acid metabolism pathways of low linolenic acid soybean on plant volatile profiles.
 - I hypothesize low linolenic acid soybean cultivars will have altered volatile profiles, and that *C. trifurcata* will be less attracted to the volatile profile of low linolenic acid cultivars.

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Table 1. Increase in aphid performance on fungal infected plants.

Plant	Fungus	Aphid	Increase (%)
<i>Betula pendula</i>	<i>Marssonina betulae</i>	<i>Euceraphis betulae</i>	47.8*
<i>Cucumis sativus</i> Systemic trial 1 ^c	<i>C. cucumerinum</i> ^a	<i>Aphis gossypii</i>	42.0** N.S. ^b
<i>Cucumis sativus</i> Systemic trial 2	<i>C. cucumerinum</i> ^a	<i>Aphis gossypii</i>	13.0** N.S. ^b
<i>Cucumis sativus</i> Localized trial 1 ^d	<i>C. cucumerinum</i> ^a	<i>Aphis gossypii</i>	-125.0**
<i>Cucumis sativus</i> Localized trial 2	<i>C. cucumerinum</i> ^a	<i>Aphis gossypii</i>	26.8**
<i>Crisium arvense</i>	<i>Puccinia punctiformis</i>	<i>Capitophoris elegani</i>	25.0***
<i>Crisium arvense</i>	<i>Puccinia punctiformis</i>	<i>Capitophoris horni</i>	300.0***
<i>Crisium arvense</i>	<i>Puccinia punctiformis</i>	<i>Uroleucon</i> sp.	300.0***

* Aphid performance measured by number of developed embryos aphid⁻¹

** Aphid performance measured by number of nymphs produced on detached leaves

*** Aphid performance measured by number of aphids present on shoots of plants

^a *Cladosporium cucumerinum*

^b Non-significant at the $P = 0.05$ level

^c Aphid performance measured on detached leaves. Uninfected leaves were taken from plants artificial inoculated with fungus six days prior.

^d Aphid performance measured on detached leaves. Infected leaves were taken from plants artificial inoculated with fungus six days prior.

Chapter Two

What is the economic threshold of soybean aphids *Aphis glycines* (Hemiptera: Aphididae) in enemy free space

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Abstract

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a serious pest of soybean in the North Central United States. Current management recommendations rely on the application of insecticides based on an economic threshold (ET) of 250 aphids per plant. Natural enemies are important in slowing the increase of aphid populations and can prevent them from reaching levels that can cause economic losses. However, biological control of *A. glycines* is inconsistent and can be affected negatively by the intensity of agricultural activity. We measured the impact of a natural-enemy-free environment on the capacity of the current ET to limit yield loss. In 2008 and 2009, caged micro-plots were assigned to one of three treatments: plants kept aphid-free (referred to as the control), plants that experienced a population of 250 aphids per plant (IPM), and plants that experienced unlimited aphid population growth (unlimited). The population growth rate of aphids in the unlimited treatment for the ten days after the application of insecticides to the IPM treatment was calculated using linear regression. The linear equation was solved to determine the mean number of days between the ET and the economic injury level (EIL) for an aphid population in absence of predators. The number of days was determined to be 6.97 ± 1.11 days. The two-year average yield for the IPM treatment was 99.93% of the control treatment. Our study suggests the current soybean aphid ET of 250 aphids per plant can effectively protect yield even if the impact of natural enemies is reduced.

Keywords: IPM, population dynamics, natural enemy

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is the leading insect pest of soybean, *Glycine max* (L.) Merrill, in the North Central United States. Soybean aphids are capable of reducing yields by up to 40% (Ragsdale et al. 2007). The current recommendation to prevent yield loss is an application of insecticide to foliage (Myers et al. 2005, Ragsdale et al. 2007) when aphid populations exceed 250 aphids per plant on more than 80% of the plants from the onset of flowering to early pod development (i.e. R1 to R5 stages) (Fehr and Caviness 1977). This value serves as an economic threshold (ET) for an economic injury level (EIL; 674 aphids per plant) that was calculated from crop values and management costs typical for soybean growers in the Midwest United States. Ragsdale et al. (2007) calculated this ET based on the population growth rate of the aphid, allowing growers at least seven days to prepare for the application of a foliar insecticide. This recommendation has been shown to reduce insecticide use and be more profitable than prophylactic management of the soybean aphid in which insecticides are applied based on the growth stage of the plant regardless of aphid population density (Johnson et al. 2009, Song and Swinton 2009).

Despite the large body of literature that indicates natural enemies regulate soybean aphid populations (Fox et al. 2004, Rutledge et al. 2004, Fox et al. 2005, Nielsen and Hajek 2005, Rutledge and O'Neil 2005, Costamagna 2006, Schmidt et al. 2007, Schmidt et al. 2008), economic outbreaks are common in North America. Why these outbreaks occur is unclear, though several factors can contribute to increasing the risk for soybean aphid outbreaks. Recent studies have shown the effects land use can have on natural enemy abundance in soybeans (Gardiner et al. 2009a) and the biological control

they provide for soybean aphids (Gardiner et al. 2009b). Landis et al. (2008) explored the impact of increased land use for corn-based biofuel production on this ecosystem service. They argued that with increased incentives for corn production, corn acreage would increase, resulting in decreased biocontrol of the soybean aphid due to a more simplified landscape (Landis et al. 2008, Gardiner et al. 2009b). Pesticide use in soybean has also increased since the introduction of the soybean aphid to the United States (NASS/USDA 1999, 2005). The primary insecticides to control the soybean aphid are broad spectrum in effect, reducing natural enemy populations along with aphid populations (Jeffries and Lawton 1984, Johnson et al. 2008, Ohnesorg et al. 2009). Removal of natural enemies from an agroecosystem can lead to rapid re-colonization by a pest and secondary outbreaks due to the creation of enemy-free space (Jeffries and Lawton 1984).

The ET developed by Ragsdale et al. (2007) for the soybean aphid was developed with at least a seven-day lag time between the ET and EIL that provides growers an opportunity to schedule an insecticide application to their fields. If aphidophagous arthropods diminish in the landscape, the growth rate of aphid populations in the field would increase. If the population growth rate increases during the critical time between 250 aphids per plant (ET) and 674 aphids per plant (EIL), the ET may need to be lowered to still provide a seven-day lag time and prevent yield loss from occurring. A recent study conducted under semi-field conditions with soybean plants artificially infested with aphids and grown within cages has suggested the ET should be reduced to as low as three aphids per plant (Catangui et al. 2009). The conditions under which the experiment was

carried out excluded the impact of aphidophagous natural enemies, creating an enemy free space. However, Catangui et al. (2009) did not compare the yield response of such a low threshold (e.g. no aphids) to that of Ragsdale et al. (2007). It is unclear if yield loss or any impact to soybean plants will occur at either density of aphids.

Considering the current simplification of the agricultural landscape and the increased use of insecticides for control of the soybean aphid it is reasonable to assume that the biological control provided by aphidophagous arthropods will diminish. Previous studies have used cages to estimate the impact of predators on soybean aphid populations (Fox et al. 2004, Schmidt et al. 2007, Gardiner et al. 2009b). By comparing the growth of aphid populations within cages to those outside cages, several studies have observed a substantial decrease in aphid population growth when predators have access to aphids. However, growth of aphid populations within cages can be affected by other factors. For example, a previous study showed that the temperature inside a cage can vary from that outside (Fox et al. 2004). Temperature can have a dramatic impact on aphid development time and population growth rates (McCornack et al. 2004) and temperature based models have been developed to predict *A. glycines* outbreaks (Venette et al. 2004).

Our goal was to evaluate the current recommended ET for the soybean aphid in a natural enemy-free environment (i.e. a cage). We hypothesized that in absence of the natural enemies, soybean aphid populations would reach the ET (250 aphids per plant) and exceed the EIL (674 aphids per plant) earlier than the seven days proposed by Ragsdale et al. (2007). We anticipated that within the cages, a temperature based model could predict when populations reached the EIL from the ET. Furthermore we

hypothesized that soybean aphid populations that reach the ET of 250 aphids per plant but do not exceed the EIL would not have an impact on the plant.

Materials and Methods

We conducted the following experiment during 2008 and 2009 at the Iowa State University Horticulture Research Station north of Ames, in Story Co. Iowa. We grew soybeans in replicated plots (28 cm by 51 cm) kept 152 cm apart within six blocks. The ground in between plots was planted to foxtail, *Setaria spp.* Foxtail was kept <0.6 m tall by mowing as needed. Plots were planted with commercially available soybean cultivars adapted for growing in the region. Five cultivars were used in 2008 with a sixth cultivar added in 2009. Six different pest treatments were established in a complete factorial design with each combination of treatment by cultivar present. A randomized complete block designed was used in 2008 with six replications. The addition of a sixth cultivar in 2009 necessitated the use of a randomized incomplete block design with six blocks containing five replications of each treatment by cultivar combination. A sub-set of these treatments by cultivar combinations is reported here. This sub-set of treatment by cultivar combinations was part of a larger experiment conducted to evaluate the impact of multiple pests on soybean cultivars differing in seed composition characteristics. For the analyses presented here, two cultivars, DK 27-52 and DK 28-52 (Monsanto Company, St. Louis, MO) and three pest treatments were included. Planting occurred on 1 June and 19 May in 2008 and 2009, respectively. Planting density was 22 seeds per plot and plants were thinned to 10 evenly spaced plants per plot after emergence.

Three aphid population levels were established and randomly assigned to plots within each of six blocks. The first level was kept free of aphids, and is referred to as the ‘control treatment’ throughout this document. The second level was a density of 250 aphids per plant, and is referred to as the ‘IPM treatment’. The final infestation level consisted of allowing aphid populations to grow without limit, and is referred to as the ‘unlimited treatment’.

To control the density of aphids within each treatment, cages were placed around plots. Cages were constructed of white no-see-um mesh fabric (Quest Outfitters, Sarasota, FL) stretched over cage frames constructed of thin-walled PVC pipe (Charlotte Pipe, Charlotte, NC). Cages measured 1.1m by 0.8m by 0.8m (height by length by width). Cages were placed over plots after planting, at the VC-V1 growth stage (Fehr and Caviness 1977), and remained until after plots were harvested. For the remainder of this document ‘cage’ will be used to refer to both the plot and the physical cage surrounding the plot.

Aphids used in this experiment came from a laboratory colony maintained at Iowa State University. The laboratory colony was established from field populations collected from central Iowa in 2004 and maintained on commercially available aphid susceptible soybean cultivars. The colony was supplemented with field populations from central Iowa each summer from 2005 to 2008.

Treatments that received aphids (IPM and unlimited) were infested by randomly selecting one plant per cage and infesting it with five soybean aphids on the second trifoliate at the V3-V4 growth stage. Initially infested plants were marked by tying a strip

of fluorescent flagging tape to the stem at soil level. Aphid infestations occurred on 3 July in 2008 and on 23 June in 2009. Aphid populations were counted twice a week by counting all aphids (immature and adult stages) on the initially infested plant. Care was taken not to damage plants during the counting of aphids. If a plant was damaged during counting it was removed from aphid growth rate analyses. The remaining plants in each cage were infested when cages reached 50 aphids on the initially infested plant. The secondary infestation was accomplished by clipping leaves with approximately 50 aphids onto the newest expanded trifoliolate. The initial infestation was followed to determine the population growth rate of aphids within the cages. The secondary infestation was performed to obtain aphid infestations that were more uniform in spatial pattern throughout a cage for the purpose of collecting yield data in response to varying aphid densities. IPM treatment cages received a single application of λ -cyhalothrin (Warrior with Zeon Technology, Syngenta Crop Protection, Greensboro, NC) when populations reached 250 aphids per plant. Insecticides were applied using a backpack sprayer and Teejet (Springfield, IL) twinjet nozzle (TJ 11002) with 20 gallons per acre at 40 pounds per square inch pressure. Nets were opened and lowered to ground level and plots were wrapped with a spray shield (117 cm x 117 cm laminated paper) during insecticide application to ensure adequate insecticide coverage and limit insecticidal drift. Immediately after insecticide application nets were raised and closed again. Populations in the unlimited treatment were counted on the initially infested plant twice each week until the populations reached over 1,000 aphids/plant. Populations were then measured once each week until all aphid populations had declined from the previous sampling date.

The effect cages had on temperature and relative humidity were measured using HOBO[®] micro stations equipped with Temperature/RH smart sensors (Onset Computer Corporation, Bourne, Massachusetts). Two stations were positioned at opposite ends of the field (37 m apart). Each station was equipped with four sensors. Each sensor recorded both temperature and relative humidity. One sensor was positioned outside of a cage. The other three sensors were placed inside the three nearest cages to the outside sensor. Sensors inside the cage were attached to one of the support legs approximately 1.0 m off the ground. Sensors recorded temperature and relative humidity every thirty minutes for the duration of the experiment.

Yield was measured by hand harvesting all ten plants in each cage. Seed was dried to a uniform moisture content to under eight percent. Total seed weight was then measured for each cage.

Data analysis

Temperature and Relative humidity

Sensors inside and outside of cages were used to collect temperature and relative humidity data starting seven days after cages were infested with aphids and until aphid populations declined. Daily maximum and minimum temperatures were used to calculate the accumulated degree-days for a single day using the following equation:

$$DD = \left(\frac{MaximumTemp. - MinimumTemp.}{2} \right) - DevelopmentalThreshold$$

In the equation the developmental threshold is set at 8.6°C and the upper developmental threshold set at 34.9°C in accordance with previous studies on soybean aphid

development (Hirano et al. 1996, McCornack et al. 2004). The degree-day equation was used as outlined by Pedigo and Rice. We summed degree-days for the entire season to calculate the cumulative degree-days from the temperatures recorded by the sensors inside and outside of cages. Average relative humidity was calculated for each day from each sensor and was used to compare the relative humidity inside and outside the cages.

Aphid Population Growth

Aphid population data from the cages assigned the unlimited treatment were used to determine how quickly soybean aphids reach the EIL from the ET in enemy free space. The effects of year and cultivar and their interaction were tested using a mixed model (PROC MIXED; SAS Institute 2001). Block was set as a random effect in the model. The rate of population growth of aphids in each cage (total of 22 cages) was estimated. The linear relationship for the density of aphids over time was estimated using regression analysis. The density of aphids was log transformed to control for heteroscedasticity. This rate of growth per day was estimated during a period of time that began when populations reached 250 aphids per plant and ended ten days later.

We used the rate of growth calculated from each cage to determine how many days were required for a population to grow from the ET to the EIL. We accomplished this by plotting the aphid density (ln aphids/plant) on the y-axis and time (in days) on the x-axis. The following equation was then solved:

$$y = mx + b$$

Where $y = \ln 674$ (the current EIL), m = population growth rate for an individual cage and $b = \ln 250$ (the current ET). The equation could then be solved for “ x ”, which gives the time it took the aphids in an individual cage to increase in density from the ET to the EIL.

Aphid Growth Model

We compared our observations of aphid population growth within cages to populations predicted by the temperature-based model Soybean Aphid Growth Estimator (SAGE) ver 1.2 (McCornack and Ragsdale et al. 2004, Venette et al. 2004) using Student’s *t*-test. The SAGE model was designed using the soybean aphid growth parameters from McCornack et al. (2004). The SAGE is available online free of charge through the University of Minnesota’s soybean extension website. The SAGE model is a management tool designed for farmers and crop advisors to predict future within field aphid population growth based on the current aphid population within a field and predicted temperatures.

The SAGE model predicts the aphid population over a seven-day period based on the daily minimum and maximum temperatures for the current day and the following seven days. To determine predicted rates of population growth, we used the daily minimum and maximum temperatures recorded from sensors inside the cages. We added temperature data from our field sensors to SAGE (Venette et al. 2004). We used temperatures from the seven-day period when the aphids in the unlimited treatment were in the range of the ET to the EIL. The initial aphid population used in this model was

250 aphids per plant. The output from the model was a daily estimate of aphid density, which was log transformed and a rate of growth was calculated. A unique rate was calculated from temperature data collected from each sensor. The average amount of time between the ET and EIL predicted by this model was estimated for each year.

Yield

We employed two soybean cultivars within each treatment. To reduce the variation in yield across these cultivars we calculated a ratio for each cultivar based on the yield measured in each treatment compared to the aphid-free control treatment. This resulted in the equation:

$$\text{Yield ratio} = \text{yield of treatment plot} / \text{yield of control plot}$$

The yield ratio analysis yielded 44 observations across the two cultivars and two aphid treatments. Four observations were not used due to missing yield data for either the treatment plot or aphid-free control plot.

The means of the ratios for the IPM and unlimited treatments are reported.

Yield ratios were compared between the IPM and unlimited treatments using an ANOVA to determine the effect of insecticide treatment on yield. Our mixed model included the fixed effects of year, treatment, cultivar and the interactions of cultivar by year, treatment by year, cultivar by treatment and the three-way interaction of cultivar by treatment by year. Block was set as a random effect. The Student's *t*-test was used to determine if the yield ratio of the IPM treatment was significantly different from "1". This comparison

determined if the aphid exposure experienced by plants in the IPM treatment was sufficient to reduce yield.

Results

Temperature and relative humidity

The average (\pm SEM) degree-days accumulated outside of the cages in 2008 and 2009 were 685 ± 3.1 and 638 ± 2.5 respectively. The average (\pm SEM) degree-days inside of cages were 703 ± 1.8 and 675 ± 7.8 in 2008 and 2009 respectively. For the two years of this study, the temperatures within the cages were higher than those outside the cages resulting in an average of 28 degree-days, or a four percent increase in degree-days experienced within the cages. The average daily relative humidity for the season in 2008 and 2009 was 81.6% and 82.8% respectively. The average daily humidity inside cages was 79.1% and 79.9% in 2008 and 2009 respectively. Overall, we measured an average reduction of 2.7% in humidity within the cages during the two years of our study.

Aphid Populations and Growth Dynamics

Aphid populations reached the ET between 22 July and 25 July in 2008 and between 23 July and 28 July in 2009 (Figure 1). This was 19 to 22 and 25 to 30 days after initial infestation in 2008 and 2009 respectively. In both years these dates occurred during the R1 to R3 growth stages of the plant. Aphid populations peaked in the unlimited treatment between 19 August and 28 August in 2008 and between 12 August and 20 August in 2009. In both years these dates occurred during the R4 to R5 growth

stages of the plant. The average peak aphid population was 7,180 aphids/plant and 9,305 aphids/plant in 2008 and 2009 respectively.

We did not observe a difference in the population growth rate of aphids across years ($F = 1.55$; $df = 1,13$; $P = 0.235$) or cultivars ($F = 0.68$; $df = 1,13$; $P = 0.4245$) or an interaction between year and cultivar ($F = 0.09$; $df = 1,13$; $P = 0.7677$). Therefore, data were pooled across years and cultivars to estimate an average growth rate. The average growth rate per cage was calculated to be 0.14 ± 0.06 ln aphids plant⁻¹ day⁻¹ with a 95% confidence interval.

The growth rate of aphids in each cage was also used to calculate the average number of days required for a population to grow from 250 aphids per plant to 674 aphids per plant. From the 19 cages used in this study, we observed populations reaching 674 in an average of 6.97 ± 1.11 days (Figure 2).

Aphid Growth Model

Daily high, low and average temperatures for the eight days in each year used in the model calculations are listed in table 1. From these temperatures we predicted the abundance of aphids (Fig. 3). The model predicted an average growth rate of 0.33 ± 0.004 ln aphids day⁻¹. This was significantly greater than our observed growth rate of 0.14 ($t = 236.79$; $df = 32$; $P = <0.0001$). The model's predicted growth rate resulted in an estimate of 2.8 and 3.3 days, respectively, for 2008 and 2009, for the time between the ET and EIL.

Yield

We report yield as the ratio of the seed weight for both the IPM and unlimited treatments to the control treatment (Figure 4). No significant interactions were present between aphid treatment and cultivar ($F = 0.42$; $df = 1,27$; $P = 0.5244$), aphid treatment and year ($F = 0.60$; $df = 1,27$; $P = 0.4448$), cultivar and year ($F = 0.04$; $df = 1,27$; $P = 0.8494$) or aphid treatment, cultivar and year ($F = 0.60$; $df = 1,27$; $P = 0.4468$). Neither cultivar ($F = 0.11$; $df = 1,27$; $P = 0.7424$) nor year ($F = 0.33$; $df = 1,27$; $P = 0.5678$) had a significant effect on yield ratios. Yields were then pooled across cultivars and years for all further analyses. We observed a significant difference of 46% between the yield ratios of the IPM and unlimited treatments ($F = 13.65$; $df = 1,27$; $P = 0.0009$) (Figure 4). We did not observe a difference in the yield ratio of the IPM treatment from a ratio of 1 ($t = 0.01$; $df = 1,27$; $P = 0.9948$), indicating that the aphid densities in the IPM treatment did not significantly effect yield (Figure 4).

Discussion

The growth of aphids was slower than what was predicted from a temperature-based model (Venette et al. 2004) of soybean aphid growth, which was created from developmental thresholds calculated by McCornack et al. (2004). McCornack et al. (2004) found the optimal temperature for development to be 27.8°C. In our study both external temperatures and internal cage temperatures oscillated above and below this threshold, often by as much as 7°C. The developmental thresholds of the soybean aphid were calculated based on the growth of populations in an environment with a constant

temperature (McCornack et al 2004). The difference in the predicted rate of soybean aphid growth to what we observed may be due to daily fluctuations of temperatures in the field which was not addressed by McCornack et al. (2004). Such fluctuations may prevent the populations from growing at their optimal rate.

This difference in the predicted versus the observed growth rate of aphid populations could be due to other abiotic factors such as rain and wind (Trumble 1982, Moran et al. 1987, Sanderson et al. 1994, Maudsley et al. 1996). Although we excluded predators and parasitoids from soybean aphids in this study, entomopathogenic fungi are a source of aphid mortality and would likely not be excluded by our cages. Such fungi have been observed to reduce soybean aphid populations in North America (Baute 2003, Rutledge et al. 2004, Nielsen and Hajek 2005). However, we focused our estimates of aphid population growth, well before populations declined. Throughout the two years of the experiment we did not observe any evidence of fungal infection in the aphid populations. Furthermore, we did not observe a significant difference in relative humidity inside the cage to that outside the cages, suggesting that the cage did not affect an abiotic factor that could promote fungal growth.

Soybean aphids have been reported to be capable of doubling populations in as little as 1.5 days (McCornack et al. 2004). Ragsdale et al. (2007) reported an average doubling time of 6.8 days for naturally occurring populations in the field. In our experiment, for population densities between the ET and EIL, we observed an average population doubling time of 4.95 days. Our temperature model predicted aphid population doubling times to be 2.13 days. Ragsdale et al. proposed that the difference in

doubling times observed in the field and those predicted by temperature models were due to “environmental resistance”. Environmental resistance includes natural enemies, weather and immigration and emigration of winged aphids.

Our study suggests that for the period of time when aphid population densities are between the ET and EIL natural-enemy-free space may more closely resemble field conditions than ideal conditions for aphids. Previous studies have shown natural enemies to have a large impact on the regulation of soybean aphid populations (Fox et al. 2004, Costamagna and Landis 2006, Schmidt et al. 2007, Gardiner et al. 2009b). In all cases, these studies focused on the growth of initial populations of aphids at low densities (1-10 aphids per plant). Our study focused on populations of over 250 aphids per plant. Our results suggest that at this point of a soybean aphid outbreak, natural enemies may not be as important a source of mortality as previously thought. Rather abiotic factors may play a larger than anticipated role in environmental resistance. The difference between the soybean aphid rates of growth we observed and the one predicted by the temperature-based model may be due to abiotic factors such as, fluctuations in temperature previously noted above, and the protection of aphids from other abiotic factors such as rainfall and wind. Further research may be necessary to explore the role of these abiotic factors in regulating aphid population dynamics at the critical time between the ET and EIL.

A growing body of literature suggests that the level of natural enemy induced mortality of the soybean aphid may be diminishing due to agricultural practices (Landis et al. 2008, Ohnesorg et al. 2009, Schmidt et al. 2010). Olson et al. (2008) reported that the most commonly used insecticides for control of soybean aphids in the Midwest

included Asana[®], Lorsban[®], Mustang[®], and Warrior[®]; all are considered broad-spectrum in nature and reduce natural enemy populations in addition to aphids (Ohnesorg et al. 2009). The research presented in this paper is the first step in analyzing how our current soybean aphid management practices will respond to the changing soybean agricultural ecosystem in the Midwestern United States.

Our findings support the use of an ET of 250 aphids per plant recommended by Ragsdale et al. (2007) and supported by Johnson et al. (2009). Our data show that under cage conditions a treatment threshold of 250 aphids per plant provides yield protection from the soybean aphid. Our linear regression analysis also demonstrated that the observed aphid population growth rates in our study provided an average seven-day lag time from the ET to the EIL. This seven-day lag time is within the range proposed by Ragsdale et al. (2007). Our analysis also indicates that abiotic factors may have a larger than expected impact on aphid population during the period between the ET and EIL and further research may be necessary to enhance our understanding of these factors.

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Table 1. Daily temperatures inside cages when *Aphis glycines* populations were between the economic threshold and economic injury level

Date	Average Daily Temp. (°C) ^a	Range (°C) ^b
2008		
25-July	22.2 ± 3.4	29.4 – 19.0
26-July	24.3 ± 5.9	33.9 – 16.0
27-July	21.8 ± 5.1	32.8 – 15.0
28-July	24.7 ± 4.4	32.3 – 21.0
29-July	24.7 ± 3.4	32.3 – 21.0
30-July	25.3 ± 4.9	32.3 – 18.2
31-July	26.0 ± 4.0	32.1 – 19.7
1-August	25.0 ± 4.1	30.3 – 17.0
2009		
27-July	21.9 ± 5.9	29.9 – 13.8
28-July	21.8 ± 5.2	28.7 – 13.3
29-July	19.9 ± 5.8	27.3 – 10.3
30-July	19.1 ± 5.3	26.7 – 11.6
31-July	19.4 ± 4.7	26.5 – 12.1
1-August	19.6 ± 4.5	25.6 – 11.0
2-August	20.3 ± 5.4	27.0 – 11.2
3-August	22.7 ± 6.0	30.7 – 14.2

^a Average daily temperature is reported ± the standard deviation, temperature was recorded every half hour

^b High and low temperatures in range are an average of six sensors for each year

Figure Captions

Figure 1. Mean aphid populations per plant throughout the two years of the experiment.

Plants were infested with five aphids at the V3-V4 growth stage. Populations were sampled twice per week until densities reached over 1,000 aphids per plant. Sampling then occurred once per week. Sampling continued until populations declined later in the season.

Figure 2. The equation for mean aphid population growth rate between the economic threshold (ET) and economic injury level (EIL) is depicted by the bold line.

Observations for when each cage reached the EIL are also depicted as “x”s. The mean time between the ET and the EIL was 6.97 ± 1.11 days.

Figure 3. Predicted aphid population growth for 7 days beginning at the density of the economic threshold (250 aphids per plant). The 2008 and 2009 models were calculated using the soybean aphid growth estimator version 1.2 developed by Venette et al. (2004) and temperature data collected from inside cages. The observed values were calculated from the population growth rates observed during the experiment.

Figure 4. Mean seed weight ratio of the two aphid treatments averaged over the two varieties and two years of the study. Significant treatment differences determined using LSMEANS are represented with letters. Significant differences between a treatment mean and a ratio of “1” determined using Student’s *t*-test are represented with an asterisk and signify a yield loss due to the treatment.

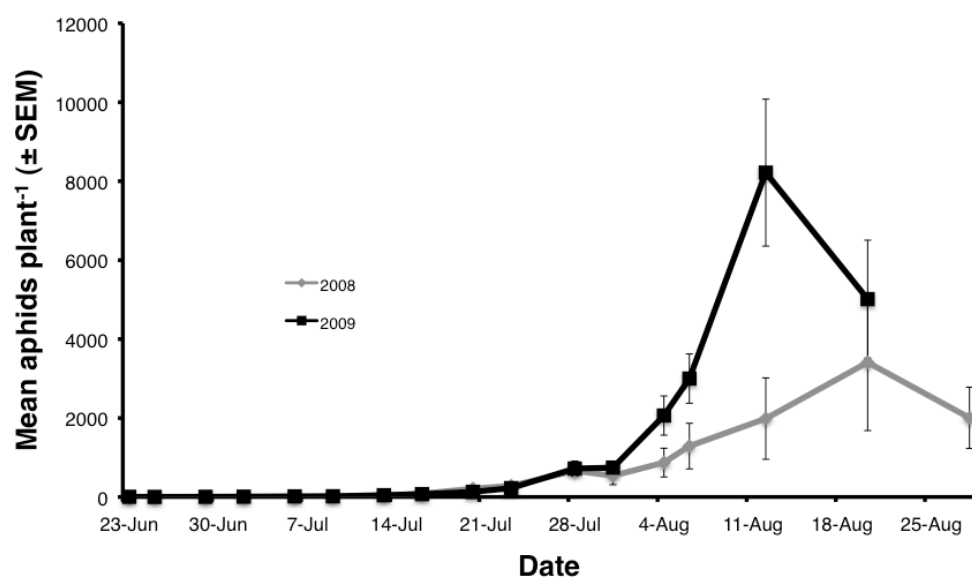
Figure 1.

Figure 2.

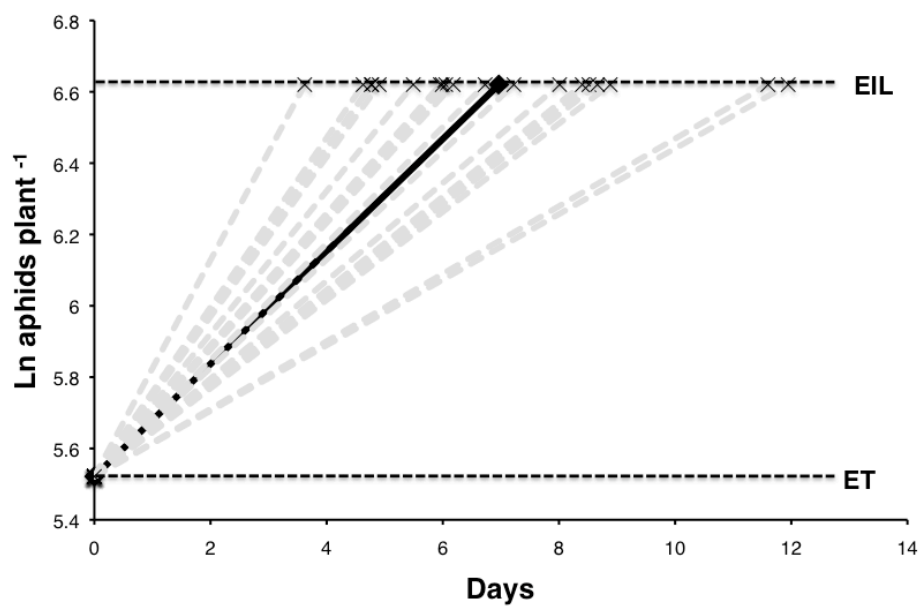


Figure 3.

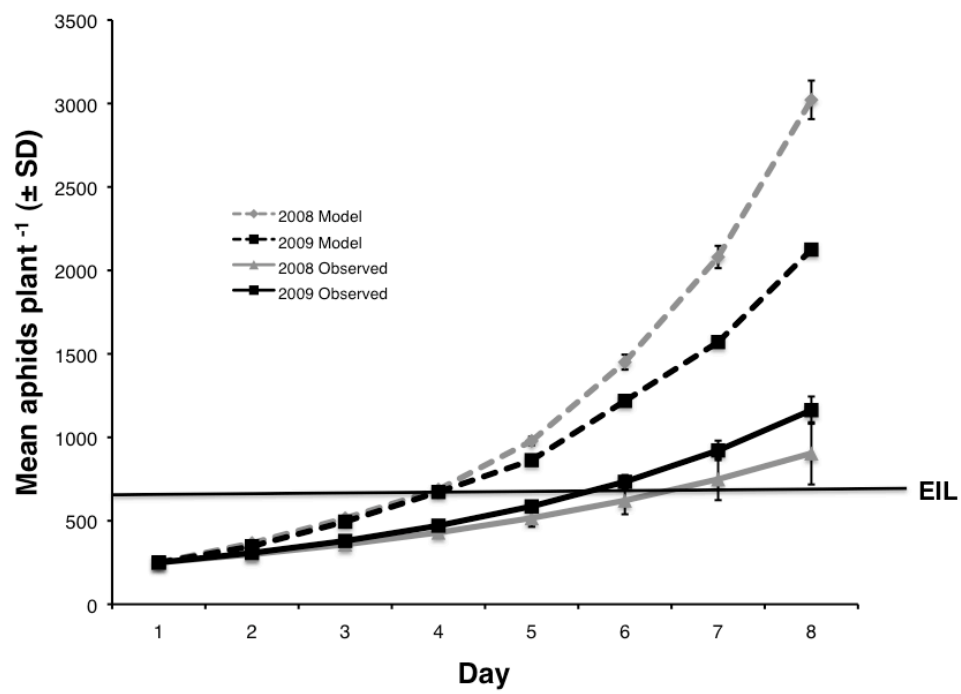
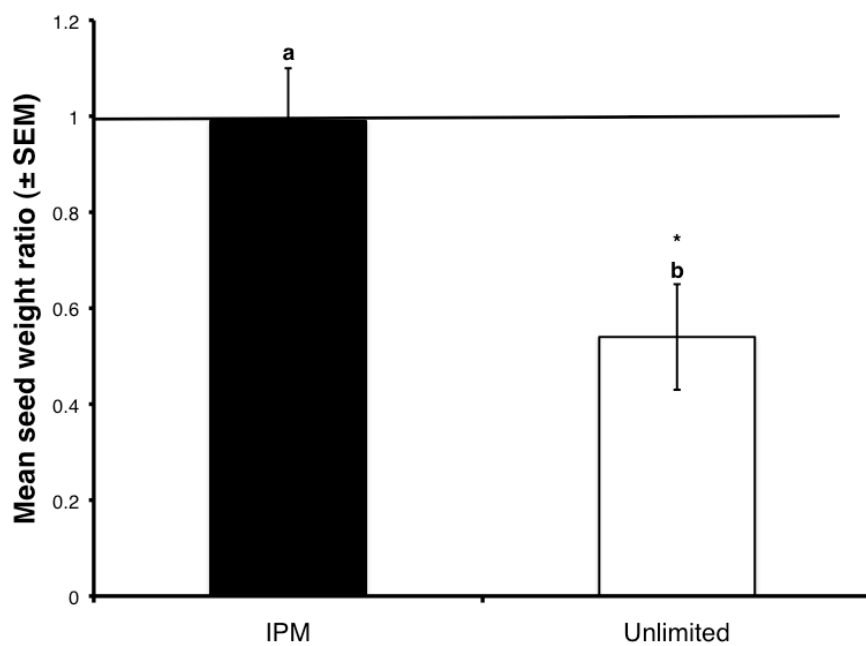


Figure 4.

Chapter Three

Interactions between a nematode-fungus disease complex and an above ground herbivore across resistant and susceptible soybean cultivars

A paper to be submitted to Ecological Applications

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Abstract

Soybean is an introduced crop to America and because of this it has benefited from a small number of pests threatening its production. Since its rapid expansion in production acres beginning in the 1930s, several pests have been introduced from the native range of soybean. Our knowledge of how these pests interact and the implications for management is limited. We examined how three common economic soybean pests, the nematode *Heterodera glycines*, the fungus *Cadophora gregata* and the aphid *Aphis glycines* interact on soybean. From 2008 to 2010 six soybean cultivars were exposed to four different pest treatments consisting of *H. glycines*, *C. gregata* and *A. glycines* in a micro-plot field experiment. Pest treatments were manipulated using artificial infestations in a field virgin to soybean production. Plots were artificially infested with either a single pest or all three pests in combination. The performance of each pest was measured in a “single pest” treatment and compared to pest performance measured in the “multiple pest” treatment. This design allowed us to measure the impact of the presence of two other soybean pests on the performance of each pest. Soil samples were taken prior to planting and after harvest to assess *H. glycines* performance during the season. Internal stem discoloration was used as a measure of *C. gregata* performance. *Aphis glycines* populations were monitored from initial infestation to leaf senescence to track performance. The presence of multiple pests significantly decreased *A. glycines* and *C. gregata* performance, but significantly increased *H. glycines* performance.

The introduction and cultivation of plants into exotic areas of production has often separated these plants from their associated herbivores and pathogens (Mack et al. 2000). When the exotic plant is reunited with a pests from its' native range, the consequences can be economically and environmentally significant (Pimentel et al. 2003). Furthermore, as exotic species accumulate, there is the potential for an 'invasional meltdown' (Simberloff and von Holle 1999) to occur, such that there is increased likelihood of survival and/or ecological impact of these species. Heimpel et al. (2010) considered soybean (*Glycines max* L.), the soybean aphid (*Aphis glycines* Matsumura), and the overwintering host of the aphid, European buckthorn (*Rhamnus cathartica* L.) part of such an invasional meltdown in North America. The intentional introduction of these host plants for *A. glycines* facilitated its establishment and regional abundance in North America. Additional factors within soybean fields may further facilitate the establishment of *A. glycines*. Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) and *A. glycines* are both pests of soybean in the North Central region of the US and originally had a neotropic distribution (Schmitt et al. 2004; Venette and Ragsdale 2004). Hong et al. (2010) observed a greater intrinsic rate of growth of *A. glycines* on soybean infected with a pathogen that is exotic to North America, *H. glycines* within a laboratory assay. However, they also observed a preference for *H. glycines* un-infected plants by alate *A. glycines*. To what extent these two invasive pests facilitate each other's growth and establishment within agroecosystems is not clear. Understanding the relationship between these two invasive pests can provide further insight into how

invasive pests can exacerbate pest management within agroecosystems.

Furthermore, if there is a positive interaction between *A. glycines* and *H. glycines*, the consequences could further exacerbate their already significant economic impact on soybean production within the US (Niblack et al. 2006; Ragsdale et al. 2011).

Heterodera glycines affects other pests of soybeans, like the fungal pathogen, brown stem rot (BSR) (*Cadophora gregata* Harrington and McNew). *Cadophora gregata* is a common fungal pathogen, present in 68-73% of soybean fields in Illinois, Iowa and Minnesota (Workneh et al. 1999). The *C. gregata* fungus infects the soybean plant through the root system and later colonizes the vascular and pith tissue of the plant impeding the movement of nutrients. Both the incidence and severity of *C. gregata* is increased when soybean is infected by *H. glycines* (Tabor et al. 2003, 2006a, 2006b). Co-infection of *H. glycines* allows earlier colonization of the soybean roots by the fungus and possibly creates entry sites for the pathogen. Current *C. gregata* management relies on cultural control methods including crop rotation and tillage. Resistant cultivars are also available for control of *C. gregata*. When *C. gregata* and *H. glycines* occur within the same field, it is recommended to plant cultivars with resistance to both pathogens. The *H. glycines* resistance source PI88788 is shown to display some resistance to *C. gregata* in the absence of *H. glycines* infection (Hughes et al. 2004). PI 88788 is the most commonly employed source of resistance, comprising over 95% of commercially available *H. glycines* resistant cultivars (Niblack et al. 2006). To what extent PI88788 confers resistance to other sources of plant stress, like *A. glycines*, is not known.

An additional issue that can exacerbate the impact of invasive pests on crop plants is the reduction in genetic diversity that is associated with domestication (Hyten et al. 2006). Domestication of crop plants may not include genes that confer resistance to pests. For example, many members of the plant family Cucurbitacea produce cucurbitacins, a group of triterpenes, that are a deterrent to insect herbivores (Tallamy et al. 1997). However, these compounds are very bitter to humans, and commercial varieties of cucurbits are bred for low concentrations of cucurbitacins within the fruit. Domestication may disrupt the multi-trophic interactions among the plant, pests and the associated natural enemies (e.g. Rasmann et al. 2005). Plant breeding that produced identity-preserved or output trait cultivars (Mazur et al. 1999) may impact components of the plant defense against pests and pathogens. For example, soybean cultivars with modified seed contents of fatty acids have been developed for improved end user traits, specifically functionality of the soybean oil (Fehr 2006). These cultivars contain mutations to one or more genes coding for omega-3 fatty acid desaturase (*FAD3*) enzymes in the microsomal fatty acid synthesis pathway (Bilyeu et al. 2005). Cultivars with levels of linolenic acid (18:3) reduced from 8% to less than 3% reduce or even eliminate the need for chemical hydrogenation. Chemical hydrogenation of soybean oil increases the stability and shelf life of the oil and leads to the formation of saturated and *trans*-fatty acids, which are linked to an increase in coronary heart disease (Department of HHS and USDA 2005, Chappel and Bilyeu 2007). Linolenic acid is also a precursor to key plant defense signaling compounds including jasmonic acid

and green leaf volatiles (Shah 2005, Matsui 2006, Smith and Boyko 2007). Jasmonic acid is important in amplifying the initial defense response to pathogens and herbivores.

The goal of this experiment was to assess the performance of the three common soybean pests (*H. glycines*, *A. glycines*, and *C. gregata*) on commercially available soybean cultivars differing in seed linolenic acid content and *H. glycines* resistance. We first hypothesized that the abundance of each pest would differ between plants exposed to one pest versus all pests combined. Second we hypothesized that soybean cultivars with modified fatty acid synthesis pathways would experience greater populations of pests. Furthermore, we hypothesized that the response of individual pests across these cultivars would vary in the presence of *H. glycines* resistance.

Materials and Methods

We conducted the following experiment in 2008, 2009 and 2010 at the Iowa State University Horticulture Research Station north of Ames in Story County IA. This research station was selected because it had not been planted to soybean for over ten years, ensuring a minimal population of soybean pathogens and pests within the soil, including *H. glycines* (Niblack et al. 2006). We used commercially available soybean cultivars (described below) adapted for growing in the region. Soybeans were grown in micro-plots (28 cm by 51cm) and exposed to varying types of pest based on the experiment design. Plots were 152 cm away from each other.

Plots were planted with 22 seeds in a single furrow. Planting occurred on 1 June in 2008 and on 19 May in 2009 and 2010. When plants produced a single trifoliate termed the first vegetative stage (V1) (Fehr and Caviness 1977) plots were thinned to 10 plants and cages (1.1 m by 0.8m by 0.8m) were placed over the plots. Cages were designed to prevent small herbivores from feeding on the plants as well as natural enemies from feeding on *A. glycines*. Cages consisted of white no-see-um mesh fabric (Quest Outfitters Sarasota, FL) stretched over PVC pipe cage frames and anchored into the soil. Cages remained over the plots for the duration of the experiment, with any weeds and foreign insects removed by hand.

Experimental design

We assessed the effect cultivars of soybeans that varied in resistance to *H. glycines* and seed linolenic acid concentration have on three pests (*H. glycines*, *A. glycines* and *C. gregata*). The experiment was designed such that there were three main factors; two factors were due to differences among the soybean cultivars (seed linolenic acid level and *H. glycines* resistance). The third main factor was the presence of a single pest or all pests combined on each cultivar. Each combination of a pest inoculum and soybean cultivar was replicated within microplots in a randomized complete block design with six blocks.

We used a total of six soybean cultivars that were commercially available to growers within Iowa. We selected cultivars that varied by the concentration of linolenic acid in harvested seed, varying from 7% (a concentration that is typical for most soybean cultivars grown for livestock consumption) to 3% and 1%. These later

two concentrations are considered low and ultra-low respectively, and are grown to produce seed for use in the production of food items for human consumption. From these cultivars we selected those that were both susceptible (SCN-S) and resistant (SCN-R) to *H. glycines* (SCN), with this resistance derived from the PI 88788 source. However, in 2008 we were unable to find a 3% cultivar that was SCN-S; a 3% SCN-S cultivar was included in 2009 and 2010. Therefore in 2008 we used five soybean cultivars and six in 2009 and 2010 (Table 1).

We created four pest treatments for each cultivar by exposing each cultivar to an individual pest and to all three pests (*H. glycines*, *A. glycines* and *C. gregata*) (Table 2). The combinations of cultivars and pest inoculums created 20 treatments in 2008 for a total of 120 microplots with six replications per treatment. In 2009 and 2010 there were 24 treatments (four more treatments as a result of the addition of the sixth cultivar) for a total of 120 microplots with five replications per treatment.

Pest Inoculations and Sampling

Heterodera glycines Plots receiving *H. glycines* were inoculated using methods modified from Tabor et al. (2006). Deviations in methods were confined to the use of a different susceptible soybean cultivar (Williams 82 in place of Kenwood 94) for inoculum production and the use of an adapted field technique for incorporating *H. glycines* into the soil. In summary, *H. glycines* were incorporated into plots as nematode eggs suspended in 50ml of water. The egg suspension was poured over the soybean seeds in furrow during planting. *H. glycines* eggs were

obtained from a lab population (HG type 0) multiplied on the public cultivar Williams 82 planted in clay pots containing a sandy soil. Eggs were gathered by separating female nematodes and cysts from the roots of infested plants with a stream of water, and then wet sieving and decanting the infested soil (Gerdemann 1955, Niblack et al. 1993). Females and cysts were recovered using a 250- μ m-pore sieve placed under an 850- μ m-pore sieve. Females and cysts were then crushed using water and a motorized pestle to release the eggs (Niblack et al. 1993). The eggs were then collected on a 25- μ m-pore sieve located under a 75- μ m-pore sieve. Centrifugal flotation was used to separate eggs from soil and plant debris (Jenkins 1964). Egg densities were then counted using direct microscope observations. A higher density of 264,025 eggs/ 50ml of water was used in 2008 to encourage establishment of *H. glycines*. Lower densities of 75,000 and 4,000 eggs/ 50ml were used to supplement *H. glycines* populations in 2009 and 2010, respectively.

Initial field populations of *H. glycines* were assessed one to three weeks prior to planting. Six soil cores (19mm in diameter by 15 to 20cm in length) were collected from the seed row of each plot. Cysts were extracted from 100cm³ subsamples with a modified semi-automatic elutriator (Byrd et al. 1976). Eggs were then released from cysts as described above. One to two weeks after harvest the same procedure was used to sample end of the season nematode populations.

Cadophora gregata We produced fungal inoculum of *C. gregata* according to the methods outlined by Tabor et al. (2003), with the exception of the use of sorghum seed in place of soybean straw. In short, *C. gregata* inoculum was

produced from a single-spore isolate of strain P113 of Genotype A of *C. gregata* (Harrington et al. 2003). Cultures were started on green bean extract medium supplemented with ampicillin at 50mg/liter. Once abundant sporulation was present, conidia were suspended in deionized water, and mixed with twice-autoclaved sorghum seed. The mixture was incubated at room temperature for 25-30 days. The mixture was dried down prior to inoculation. In 2008 plots were inoculated by spreading 40g of infested sorghum seed directly into the furrow at the time of planting. In 2009 and 2010 the inoculum was mixed into the topsoil throughout the plot 24 hours prior to planting.

Disease severity was assessed each year after harvest by measuring internal stem discoloration (Tabor et al. 2006c). Internal stem discoloration is characteristic of brown stem rot disease, however other pathogens are also capable of displaying similar discoloration of the vascular and pith tissues (Allington and Chamberlain 1948, Workneh et al. 1999). All soybean stems were cut at ground level and brought back to the lab. Stems were split longitudinally and internal stem discoloration was visually assessed. Discoloration was assessed as the presence of any dark brown coloration on the vascular tissue or pith. The number of nodes discolored on the plant was assessed as a measurement of the severity of infection.

Aphis glycines A laboratory colony of *A. glycines* maintained at Iowa State University was used for all infestations. The colony was established from field-collected *A. glycines* found in multiple soybean fields in Jasper and Story counties in Iowa during 2007, with additional field-collected aphids added in 2008 and in 2009

from Story and Jasper counties. The colony was maintained on an *A. glycines* susceptible commercial cultivar, Prairie Brand 2636NRR in a growth chamber under a 14:10 day-night cycle.

Plots receiving an *A. glycines* pest treatment received an initial infestation of five aphids on a single plant. The five aphids were transferred to the upper-most expanded trifoliate at the V3-V4 growth stage which occurred on 3 July in 2008, 23 June in 2009, and 24 June in 2010. The remaining plants in each plot were infested with aphids from the laboratory colony when plots reached 50 aphids on the initially infested plant. The secondary infestation was accomplished by clipping leaves of approximately 50 aphids onto the newest expanded trifoliate. All aphids (all growth stages of both alate and apterous) were counted. Populations were counted on the initially infested plant twice each week until populations reached over a thousand aphids per plant. Populations were then counted once per week until the populations declined. Populations were considered in decline if the density decreased for two consecutive sampling dates.

Statistical Analyses

We used the sampling data for each pest to determine if the abundance of each pest varied across the different cultivars by either seed linolenic acid level or nematode resistance. We also determined if the presence of other pests affected the abundance of any one pest by comparing the abundance on cultivars infected with one or all pests. The parameters used to estimate pest abundance varied by pest. In general, pest abundance data were analyzed using the PROC Mixed procedure (SAS

REF). A split-plot model was used to analyze the data, as pest treatment was not re-randomized to plots each season. The main plot effect was pest treatment (single or multiple pest) and the split-plot effect was cultivar. Each cultivar was a unique combination of the effects linolenic acid level and nematode resistance. Fixed effects used in the model were block, pest treatment, linolenic acid level, and nematode resistance. The two and three-way interactions of pest treatment, linolenic acid level, and nematode resistance were also treated as fixed effects. The random effects statement included the variables plot (block*pest treatment) and year. This split-plot model was used to determine if cultivars of varying linolenic acid and nematode resistance levels had a variable impact on pest abundance. This general model was also used to determine if pest abundance varied between treatments with only one pest and all pests combined. An explanation for how abundance was measured is provided for each pest.

***Heterodera glycines* reproduction** We calculated an *H. glycines* reproduction factor (RF) to determine if *H. glycines* reproduction varied across cultivars or pest treatments. We calculated a reproduction factor by measuring the number of eggs per 100 cm³ soil at planting (Pi) and at harvest (Pf) for each plot. An RF was then calculated for each plot as $Pf/(Pi + 1)$. Reproduction factors were log transformed to ensure heteroscedasticity

***Cadophora gregata* disease** Stem disease ratings were averaged across all plants within a plot. Plot means were log transformed to ensure heteroscedasticity of the data.

***Aphis glycines* performance** We calculated the seasonal exposure of plants to aphids to estimate soybean aphid abundance. Seasonal exposure was measured based on the number of aphids per plant between two sampling points and is reported as cumulative aphid days (Hanafi et al. 1989). This measurement has been shown to have a strong correlation with soybean plant performance and yield (Ragsdale et al. 2007).

Soybean aphid growth was also sub-divided into three components, colonization of the plant, exponential population growth and peak abundance. This was done to further characterize the effect of soybean cultivars and pest treatment on *A. glycines* populations. The three components of *A. glycines* population dynamics were analyzed separately using the mixed model.

After infestation, initial aphid populations (five aphids per plant) were observed to undergo sporadic increases and decreases from one time point to the next (ie. increasing to eight aphids per plant and then decreasing to three aphids per plant). Observations of aphid sampling data indicated populations of at least ten aphids per plant rarely decreased from one sampling point to the next. Based on these observations the density of ten aphids per plant was selected as an indicator of successful colonization of the soybean plant. Days to colonization of the plant was then measured as the length of time in days for an initial aphid population to reach a density of ten aphids or greater per plant.

The exponential population growth rate was analyzed by log transforming aphid densities. Transformed aphid densities were then graphed over time to form

a population growth curve. The linear rate of growth was then measured as the slope of the line for the linear phase of the growth curve. A separate linear rate of growth was calculated for each plot.

The third component of aphid population dynamics analyzed was peak aphid abundance. Peak aphid abundance was measured as the greatest number of aphids per plant that each plot reached in a single growing season. This value was log transformed to meet the assumption of heteroscedasticity.

Results

Heterodera glycines

For plots not artificially infested with *H. glycines*, soil samples revealed nematode egg densities never exceeded 500 eggs per 100 cc of soil. Those plots where egg counts were not zero did not show increases in egg densities from spring samples to fall samples. This indicates that the eggs present in these plots were not able to successfully reproduce on soybean and thus were likely not *H. glycines* eggs, but eggs belonging to another species of cyst nematode. End of season nematode egg densities (eggs per 100cc of soil) for *H. glycines* treated plots averaged (\pm SEM) 843 ± 230 , $2,335 \pm 405$, and 688 ± 147 for 2008, 2009 and 2010, respectively.

We did not observe a significant interaction among the main effects (linolenic acid level, pest treatment or nematode resistance) on nematode reproduction. We did not observe a difference in nematode reproduction across varying levels of seed linolenic acid concentration ($F = 0.47$; $df = 2,108$, $P = 0.6253$). The presence of

nematode resistance ($F = 5.12$, $df = 1,108$, $P = 0.0256$) and the presence of other pests ($F = 7.08$, $df = 1,53$, $P = 0.0103$) significantly impacted nematode reproduction (Figure 1). Nematode reproduction was 5 times greater on nematode susceptible cultivars compared to resistant cultivars. Nematode reproduction was also found to be 5.1 times greater in the multiple pest treatment compared to the *H. glycines* alone treatment.

Cadophora gregata

Stem disease ratings across three years and all plots kept free of *C. gregata* inoculum averaged 1.05 ± 0.11 (SEM). Stem disease ratings for across all three years and all plots receiving *C. gregata* inoculum averaged 2.94 ± 0.23 . This represents an approximately three-fold increase in the severity of stem disease in plots receiving *C. gregata*. It is reasonable to assume then that the browning of pith tissue was an effective indicator of the presence of infection by *C. gregata*.

A significant interaction between linolenic acid level and nematode resistance ($F = 146.33$; $df = 2,108$; $P = <0.0001$) was observed. The stem disease rating data were then analyzed separately for nematode resistant and nematode susceptible cultivars. For nematode resistant cultivars we did not observe an effect of pest treatment ($F = 0.55$; $df = 1,40$; $P = 0.4628$) or an interaction between pest treatment and linolenic acid level ($F = 2.37$; $df = 2,44$; $P = 0.1055$) on stem disease ratings. For nematode resistant cultivars, the stem disease ratings varied by linolenic acid level ($F = 79.18$; $df = 2,44$; $P = <0.0001$). This variation was due to an 89.4% reduction in disease severity in the 3% linolenic acid cultivar (Figure 2).

Stem disease ratings for nematode susceptible cultivars varied by linolenic acid level ($F = 65.54$; $df = 2,38$; $P < 0.0001$) and pest treatment ($F = 6.19$; $df = 1,34$; $P = 0.0179$). The variation across linolenic acid levels was due to a 141% increase in disease severity on the 3% linolenic acid cultivar. Disease ratings were significantly higher in the *C. gregata* alone treatments compared to the multiple pest treatments. The interaction between the effect of linolenic acid level and pest treatment was not significant ($F = 0.89$; $df = 2,38$; $P = 0.4203$) (Figure 2).

***Aphis glycines* performance**

We observed significant effects of nematode resistance ($F = 4.27$; $df = 1,107$; $P = 0.0412$), and pest treatment ($F = 12.99$; $df = 1,53$; $P = 0.0007$) on seasonal exposure of plants to aphid (i.e CAD). The effect of linolenic acid level was non-significant ($F = 2.15$; $df = 2,107$; $P = 0.1211$). None of the interactions among linolenic acid level, nematode resistance or pest treatment were significant. This analysis revealed an overall effect of plant exposure to aphids being significantly reduced in the multiple pest treatment and on SCN-R cultivars. Plant exposure to aphids was reduced by 26.4% in the presence of *H. glycines* and *C. gregata*. Nematode resistant cultivars reduced plant exposure to aphids by 19.8% (Figure 3).

Cumulative aphid days are a measurement of aphid performance across the entire growing season. We analyzed aphid performance during three parts of the season to further investigate the impacts of multiple pests and nematode resistance on *A. glycines* performance. We investigated early season aphid performance by analyzing days to successful plant colonization. Middle of the season aphid

performance was investigated by analyzing population growth rate and end of the season performance was investigated by analyzing peak aphid abundance.

Further analyses of the effect of nematode and pest treatment on the three components of *A. glycines* population dynamics showed inconsistent results across years. In 2010, there were no significant effects on any of the three growth parameters we measured. Analyses on the days required for colonization to occur (Table 4) found significant effects in only 2008. In 2008, there was a significant effect of nematode resistance ($F = 5.29$, $df = 1,45$, $P = 0.0261$). There was also a significant interaction between linolenic acid level and nematode resistance ($F = 4.21$, $df = 1,45$, $P = 0.0460$). In 2009, the effect of nematode resistance ($F = 0.55$; $df = 1,43$; $P = 0.4607$) and the interaction of linolenic acid level and nematode resistance ($F = 0.75$; $df = 2,43$; $P = 0.4803$) were non-significant.

Analysis of aphid growth rates (Table 5) revealed a significant effect of pest treatment ($F = 10.82$, $df = 1,45$, $P = 0.0020$) and a significant interaction of pest treatment and linolenic acid level ($F = 4.30$, $df = 2,45$, $P = 0.0195$) in 2008. In 2009 the interaction of linolenic acid level and nematode resistance was significant ($F = 4.77$, $df = 2,43$, $P = 0.0134$). However, in 2009 the effect of pest treatment ($F = 1.42$; $df = 1,43$; $P = 0.2394$) and the interaction of pest treatment and linolenic acid level were non-significant ($F = 0.55$; $df = 1,43$; $P = 0.4607$).

Peak aphid abundance analyses (Table 6) revealed the effect of pest treatment, to be significant in both 2008 ($F = 5.87$, $df = 1,45$, $P = 0.0195$) and 2009 ($F = 1.39$, $df = 1,39$, $P = 0.0004$). In both 2008 and 2009, lower peak aphid densities

were reached in the multiple pest treatment when compared to the *A. glycines* alone treatment. The effect of pest treatment ($F = 1.51$; $df = 1,43$; $P = 0.2264$) was non-significant in 2010. The interaction of linolenic acid level and nematode resistance was significant in 2009 ($F = 5.64$; $df = 1,43$; $P = 0.0067$), but not in 2008 ($F = 2.04$; $df = 1,43$; $P = 0.1599$) or 2010 ($F = 0.64$; $df = 1,43$; $P = 0.5317$).

Discussion

Our study sought to investigate our hypotheses that altering seed linolenic acid levels of soybean would impact the performance soybean pests and pathogens and that soybean pests would be able to interact indirectly through a shared host plant. We tested the performance of three common Midwestern soybean pests and pathogens; *H. glycines*, *A. glycines* and *C. gregata*. Across three growing seasons and multiple performance metrics we found no support for our first hypothesis that altering seed linolenic acid levels would impact pest performance. We did however discover both positive and negative indirect interactions between the pests and pathogens in our study. We also uncovered a previously unreported effect of the commonly deployed soybean resistance source PI 88788.

Analysis of *A. glycines* performance revealed a negative impact of subsequent soybean plant colonization by *H. glycines* and *C. gregata* (Figure 3). Our experimental design does not allow us to isolate the individual effects of *H. glycines* colonization and *C. gregata* infection on *A. glycines* performance. However, previous literature may suggest possible explanations for our results. Negative impacts of

nematode colonization on aphid performance have been reported in other systems (Bezemer et al. 2005, Kaplan et al. 2009, Vandegehuchte et al. 2010). In the soybean system, Hong et al. (2010) found evidence for an influence of *H. glycines* colonization on *A. glycines* alatae host plant preference. In a laboratory study they found alates preferred control soybean plants when paired with soybean plants colonized by *H. glycines*. This preference however was not mirrored in performance, as *A. glycines* performed equally on *H. glycines* colonized plants and control plants. In their study *A. glycines* performance was measured in the laboratory under controlled conditions for a one-week period. Our study may point to a negative impact of *H. glycines* colonization on season long aphid performance as the cause for alatae avoidance of *H. glycines* colonized plants.

Heterodera glycines population data revealed an unexpected effect of pest treatment on *H. glycines* reproduction. We observed significantly elevated *H. glycines* reproduction in the presence of simultaneous *C. gregata* and *A. glycines* infestation (Figure 1). Again, our experimental design does not allow us to isolate the individual effect each pest has on *H. glycines* reproduction. In the literature there are several studies that have investigated the positive impact *H. glycines* colonization has on *C. gregata* colonization and growth. These studies have shown that *H. glycines* colonization increases both the incidence and severity of *C. gregata* infection in both *C. gregata* susceptible and resistant cultivars. We, however, are unaware of any studies that have investigated the reciprocal effect of *C. gregata* colonization on *H. glycines* performance.

It is also possible that *A. glycines* colonization may alter *H. glycines* reproduction through indirect impacts on host plant metabolism and defenses. We are also unaware of any studies that have shown a positive impact of aphid colonization on nematode performance. Previous studies have shown *A. glycines* colonization can have systemic impacts on soybean plant metabolism. It is possible that these effects may improve the host quality of soybeans through either improved nutritional quality or suppressed plant defenses. Recent research, has shown multiple aphid species in different systems capable of suppressing effective plant defenses and manipulating primary host metabolism to improve the nutritional quality of the host plant (Goggin 2007, Will et al. 2007, Walling 2008, Giordanengo et al. 2010).

Our study also examined the impact of the resistance source PI 88788 on the performance of all three pests in our study. Previous studies on PI 88788 derived cultivars and experimental lines have found PI 88788 to provide major resistance genes for *H. glycines* and minor resistance genes for *H. glycines* (Glover et al. 2004), *C. gregata* (Patzoldt et al. 2005) and soybean mosaic virus (Gunduz et al. 2004). The resistance source PI 88788 is used extensively in the Midwest due to its resistance to *H. glycines*. More recent research has also shown cultivars possessing PI 88788 in their background to display resistance to *C. gregata* in the greenhouse and field that equals or exceeds standard *C. gregata* resistant cultivars (Hughes et al. 2004). Our study found resistant cultivars to significantly impair the reproduction of *H. glycines*. Our results for *C. gregata* internal stem disease ratings were inconsistent. The

significant interaction between linolenic acid level and nematode resistance signifies an effect of cultivar on disease severity. The genetic background of each cultivar was not controlled. Therefore, effects other than nematode resistance and the linolenic acid level of each plant may impact the performance of the pests tested here. The variation in pest performance due to the genetic background of each cultivar is measured in the interaction between the main effects of nematode resistance and linolenic acid level. Since the effect of linolenic acid level did not show a consistent pattern in either the nematode resistant or susceptible cultivars (Figure 2), this suggests an effect of the different genetic backgrounds of each cultivar.

In nematode susceptible cultivars there was a significant effect of pest treatment on *C. gregata* disease levels (Figure 2). Previous studies have found plants co-infected with *C. gregata* and *H. glycines* to display increased disease incidence and severity. This observation was found in both *C. gregata* susceptible and resistant cultivars and *H. glycines* susceptible and resistant cultivars. Therefore, for the nematode susceptible cultivars, the decrease in disease caused by *C. gregata* in the multiple pest treatment in our study may be due to an *A. glycines* colonization induced modification of the interaction between *C. gregata* and *H. glycines*. The lack of an increase in disease may also be due to the subjective nature of our *C. gregata* disease rating process. It may be that our internal stem disease rating process is not sensitive enough to detect differences between plants co-infected with *C. gregata* and *H. glycines* and those infected with *C. gregata* alone. This may be supported by

our inability to detect differences in disease severity between nematode susceptible and resistant cultivars. Hughes et al. (2004) found foliar disease ratings to be more effective than internal stem disease ratings as indicators of resistance expression.

Our cumulative aphid day analysis revealed a negative effect of resistant cultivars on *A. glycines* performance (Figure 3). Our study is the first report on the effect of PI 88788 resistance on *A. glycines* performance. The level of *A. glycines* resistance expressed by the cultivars containing PI 88788 in this study does not appear to be practically useful as a single gene management tool. Previous work characterizing resistance levels in *H. glycines* resistant cultivars have found variation among PI 88788 cultivars (Tylka unpublished data). It is therefore possible that variations in *A. glycines* resistance levels may also be present in PI 88788 cultivars. Higher levels of *A. glycines* resistance may then be contained in other PI 88788 cultivars.

Aphis glycines resistant cultivars have recently been released commercially in the United States. These cultivars contain the single dominant *Rag1* gene providing effective control of *A. glycines* (Hill et al. 2006b, Kim and Diers 2009, Mardorf et al. 2010). Other resistance genes have already been identified and are being evaluated and introduced into commercial soybean lines (Mian et al. 2008, Zhang et al. 2009, Zhang et al. 2010). *Aphis glycines* biotypes capable of overcoming these resistance genes have already been identified (Kim et al. 2008, Hill et al. 2010). Recent research conducted by Wiarda et al. (2011) has demonstrated the potential of pyramiding the *Rag1* and *Rag2* genes for improved *A. glycines* resistance. The low-

level resistance observed in this experiment may indicate the presence of a minor *A. glycines* resistance gene in PI 88788. The presence of a minor resistance gene in PI 88788 resistant cultivars would provide a useful gene for pyramiding with current *A. glycines* resistance genes. A minor resistance gene from PI 88788 would be an excellent candidate for improving current *A. glycines* resistant cultivars since PI 88788 derived cultivars are already widely grown in the North Central United States for control of *H. glycines* and *C. gregata*. Future research will need to focus on the characterization of the *A. glycines* resistance observed here and an assessment of its usefulness in pyramiding with major genes for *A. glycines* resistance.

Aphis glycines is a newly invasive species to North America. In North America it will encounter a new suite of soybean pests and pathogens with which to interact with both directly and indirectly. Understanding the interaction of pests and pathogens is paramount to developing successful integrated management plans. *Aphis glycines* may pose a unique challenge in understanding pest interactions. Multiple studies have shown the ability of both *A. glycines* and aphids at large to alter host plant metabolism, defense and physiology. Understanding this ability and its ramifications will be important for predicting and understanding the outcome of pest interactions in the future.

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Table 1. Soybean cultivars exposed to varying pest treatments from 2008-2010.

Cultivar	Linolenic Acid Content ^a	Nematode Resistance ^b
DK 28-52	7%	Susceptible
DK 27-52	7%	PI 88788
IA 3018 ^c	3%	Susceptible
AG 2821 V	3%	PI 88788
IA 3041	1%	Susceptible
IA 3028	1%	PI 88788

^a Linolenic acid seed content as measured as percentage of whole oil content.

^b Resistance to soybean cyst nematode, *Heterodera glycines*.

^c IA 3018 not available in 2008, but added to experiment for 2009 and 2010.

Table 2. Pest treatments cultivars were exposed to from 2008-2010.

Pest Species	Area Colonized	Genotype or Phenotype Used
Soybean cyst nematode (SCN)	Root cells	0 ^a
Brown Stem Rot (BSR)	Vascular Pith Tissue	A ^b
Soybean Aphid (SBA)	Leaves and Shoots	1 ^c
SCN, BSR and SBA	Roots, Pith, Leaves and Shoots	0, A, 1

^a HG type 0 signifies susceptibility to all known soybean cyst nematode resistance sources.

^b More aggressive strain of BSR, capable of producing foliar symptoms.

^c Obtained from Iowa State University Soybean Entomology laboratory colony avirulent to both *Rag1* and *Rag2* *A. glycines* sources of resistance.

Table 3. Significant factors affecting pest densities.

Pest			
Effect	F- value	df	P-value
<i>Heterodera glycines</i> ^a			
Pest treatment ^b	7.08	1,53	0.0103
Nematode resistance ^c	5.12	1,108	0.0256
<i>Cadophora gregata</i> ^{d,e}			
SCN ^f -Resistant cultivars			
Linolenic acid level ^g	79.18	2,44	<0.0001
SCN-Susceptible cultivars			
Linolenic acid level	65.54	2,38	<0.0001
Pest treatment	6.19	1,34	0.0179
<i>Aphis glycines</i> ^h			
Pest treatment	12.99	1,53	0.0007
Nematode resistance	4.27	1,107	0.0412

^a Pest density assessed by calculation of a reproduction factor (RF).

^b Two pest treatments included in the analysis of each pest's density; a single pest alone and multiple pest treatment consisting of all three pests.

^c Three *H. glycines* resistant and three susceptible cultivars were used in this experiment. All nematode resistant cultivars were contained the PI 88788 derived resistance.

^d Pest density assessed by internal stem disease ratings.

^e Initial analyses of *C. gregata* densities revealed a significant interaction between linolenic acid level and nematode resistance. Further analyses were then done separately for SCN-resistant and SCN-Susceptible cultivars.

^f *Heterodera glycines* (SCN)

^g Seed linolenic acid level as measured as percentage of total seed oil. Two cultivars were tested at each of three linolenic acid levels (1%, 3% and 7%).

^h Pest density measured as cumulative aphid days (CAD) a measure of the plant's total exposure to aphids throughout the season (Hanafi et al. 1989).

Table 4. Days to Successful Colonization displayed as Mean (SEM) for 2008-2010.

Year	2008		2009		2010	
Variety	<i>A. glycines</i> ^b	Multi ^c	<i>A. glycines</i>	Multi	<i>A. glycines</i>	Multi
1% Linolenic Acid ^d						
Resistant ^e	11.5 (1.2) ^g	11.2 (3.4)	9.6 (2.6)	13.6 (3.5)	9.2 (3.3)	6.0 (1.3)
Susceptible ^f	9.7 (0.7)	11.0 (1.5)	15.2 (2.8)	10.4 (2.7)	7.4 (1.3)	8.2 (1.8)
3% Linolenic Acid						
Resistant	11.0 (1.5)	9.7 (1.4)	14.4 (1.5)	15.2 (2.1)	9.4 (0.6)	7.4 (2.6)
Susceptible			13.6 (3.5)	15.0 (3.8)	6.2 (0.8)	5.4 (1.0)
7% Linolenic Acid						
Resistant	9.8 (1.1)	13.8 (1.1)	16.0 (3.8)	16.0 (2.2)	8.0 (1.4)	5.4 (1.0)
Susceptible	8.2 (1.6)	7.5 (0.7)	13.8 (2.0)	14.4 (3.1)	7.4 (1.3)	8.2 (1.8)

^a Successful colonization was determined as ≥ 10 aphids/plant on the initially infested plant. Initially infested plants received five aphids at the V2 to V3 growth stage. Populations were counted every three to four days after initial infestation. Plants with fewer than five aphids were re-infested up to five aphids. The nematode resistant cultivars significantly increased the days to colonization in 2008 ($P = 0.0261$). The interaction of nematode resistance and linolenic acid level was also significant in 2008 ($P = 0.0460$).

^b *Aphis glycines* alone treatment. *A. glycines* were introduced at the V2 to V3 growth stage.

^c Multiple pest treatment. Plots were infested with *Heterodera glycines* and *Cadophora gregata* at planting each year. *Aphis glycines* were introduced at the V2 to V3 growth stage.

^d Linolenic Acid level in seed as percentage of total seed oil.

^e Resistance to *Heterodera glycines* derived from PI 88788.

^f Susceptible to *Heterodera glycines*.

^g Mean and (standard error).

Table 5. *Aphis glycines* population growth rates^a displayed as Mean (SEM) for 2008-2010.

Year	2008		2009		2010	
Variety	<i>A. glycines</i> ^b	Multi ^c	<i>A. glycines</i>	Multi	<i>A. glycines</i>	Multi
1% Linolenic Acid^d						
Resistant ^e	0.20 (0.01) ^g	0.16 (0.01)	0.17 (0.01)	0.17 (0.01)	0.23 (0.01)	0.20 (0.02)
Susceptible ^f	0.20 (0.01)	0.18 (0.01)	0.15 (<0.01)	0.16 (0.01)	0.20 (0.02)	0.20 (0.02)
3% Linolenic Acid						
Resistant	0.22 (0.02)	0.16 (0.01)	0.15 (0.02)	0.15 (0.01)	0.23 (0.02)	0.21 (0.02)
Susceptible			0.18 (0.01)	0.18 (0.01)	0.20 (0.03)	0.21 (0.02)
7% Linolenic Acid						
Resistant	0.18 (0.02)	0.20 (0.02)	0.16 (0.02)	0.15 (0.01)	0.20 (0.03)	0.18 (0.02)
Susceptible	0.18 (0.01)	0.16 (0.01)	0.18 (0.01)	0.15 (0.01)	0.19 (0.03)	0.22 (0.03)

^a Growth rates were determined by log transforming aphid densities and regressing them against days after infestation. Growth rates were determined as the slope for the linear phase of the regression. In 2008 the effect of pest treatment ($P = 0.0020$) and the interaction of pest treatment and linolenic acid level ($P = 0.0195$) significantly affected *A. glycines* population growth rates. In 2009 the interaction of linolenic acid level and nematode resistance ($P = 0.0134$) significantly affected population growth rates.

^b *Aphis glycines* alone treatment. *Aphis glycines* were introduced at the V2 to V3 growth stage.

^c Multiple pest treatment. Plots were infested with the *Heterodera glycines* and *Cadophora gregata* at planting each year. *Aphis glycines* were introduced at the V2 to V3 growth stage.

^d Linolenic Acid level in seed as percentage of total seed oil.

^e Resistance to *Heterodera glycines* derived from PI 88788.

^f Susceptible to *Heterodera glycines*.

^g Mean and (standard error)

Table 6. Peak aphid abundance per plant displayed as Mean (SEM) for 2008-2010.

Year	2008		2009		2010	
Variety	<i>A. glycines</i> ^a	Multi ^b	<i>A. glycines</i>	Multi	<i>A. glycines</i>	Multi
1% Linolenic Acid^c						
Resistant ^d	5951 (1842) ^f	3921 (871)	6049 (1742)	6294 (1815)	2082 (443)	2034 (489)
Susceptible ^e	10158 (2556)	5630 (1216)	2477 (676)	8333 (2600)	2342 (435)	1782 (304)
3% Linolenic Acid						
Resistant	5699 (678)	4194 (803)	1478 (289)	8419 (5579)	1620 (208)	1656 (60)
Susceptible			3600 (946)	16400 (6980)	2348 (579)	1674 (286)
7% Linolenic Acid						
Resistant	6355 (2325)	5074 (782)	2480 (493)	6400 (2123)	1662 (77)	1820 (332)
Susceptible	8189 (3405)	3928 (997)	3952 (983)	6339 (2767)	1970 (548)	2020 (642)

^a*Aphis glycines* alone treatment. *Aphis glycines* were introduced at the V2 to V3 growth stage. Peak aphid abundance was significantly reduced in the multiple pest treatment in both 2008 ($P = 0.0195$) and 2009 ($P = 0.0004$). In 2009 the interaction of linolenic acid level and nematode resistance also significantly impacted peak aphid abundance ($P = 0.0067$).

^b Multiple pest treatment. Plots were infested with the *Heterodera glycines* and *Cadophora gregata* at planting each year. *Aphis glycines* were introduced at the V2 to V3 growth stage.

^c Linolenic Acid level in seed as percentage of total seed oil.

^d Resistance to *Heterodera glycines* derived from PI 88788.

^e Susceptible to *Heterodera glycines*.

^f Mean and (standard error)

Figure Captions

Figure 1. Mean reproduction factors averaged over the *Heterodera glycines* susceptible and resistant cultivars for the three years of the study. Nematode resistance ($P = 0.0256$) and pest treatment ($P = 0.0103$) were found to be significant factors impacting *H. glycines* reproduction. The interaction between nematode resistance and pest treatment was non-significant ($P = 0.2332$). Letters represent significant differences at the $P = 0.05$ level. The *Heterodera glycines* alone treatment was infested with SCN eggs applied in the seed furrow at planting each year. The multiple pest treatment was infested with *H. glycines* and *Cadophora gregata* at planting and *Aphis glycines* at the V2 growth stage.

Figure 2. Mean internal stem disease ratings for nematode resistant and susceptible cultivars are averaged across the three years of the study. Mean stem disease ratings are reported for the *Cadophora gregata* alone treatment and the multiple pest (multiple) treatment. Stem disease ratings were assessed by counting the number of nodes per plant displaying characteristic browning of the pith tissue. Letters represent significant differences at the $\alpha = 0.05$ level. For susceptible and resistant cultivars the effect of linolenic acid level was significant ($P < 0.0001$). The effect of pest treatment was significant for susceptible cultivars ($P = 0.0179$).

Figure 3. Mean cumulative aphid days averaged across *Heterodera glycines* susceptible and resistant cultivars and the three years of the study are displayed. Lower-case letters represent significant differences at the $P = 0.05$ level between *H.*

glycines resistant and susceptible cultivars within a pest treatment. Capitalized letters represent significant differences at the $P = 0.05$ level between pest treatments averaged across both resistant and susceptible cultivars. The effects of nematode resistance ($P = 0.037$) and pest treatment ($P = 0.0007$) were significant. The interaction between nematode resistance and pest treatment was non-significant ($P = 0.6100$).

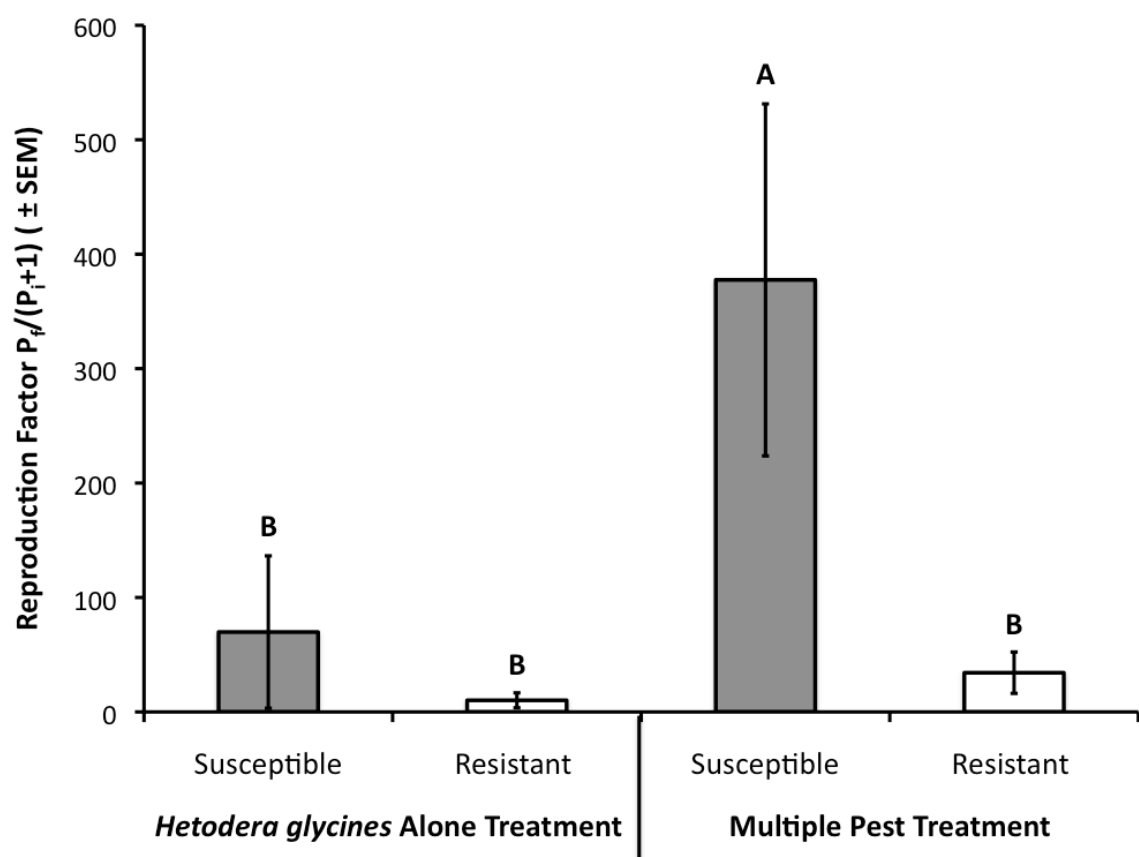
Figure 1.

Figure 2.

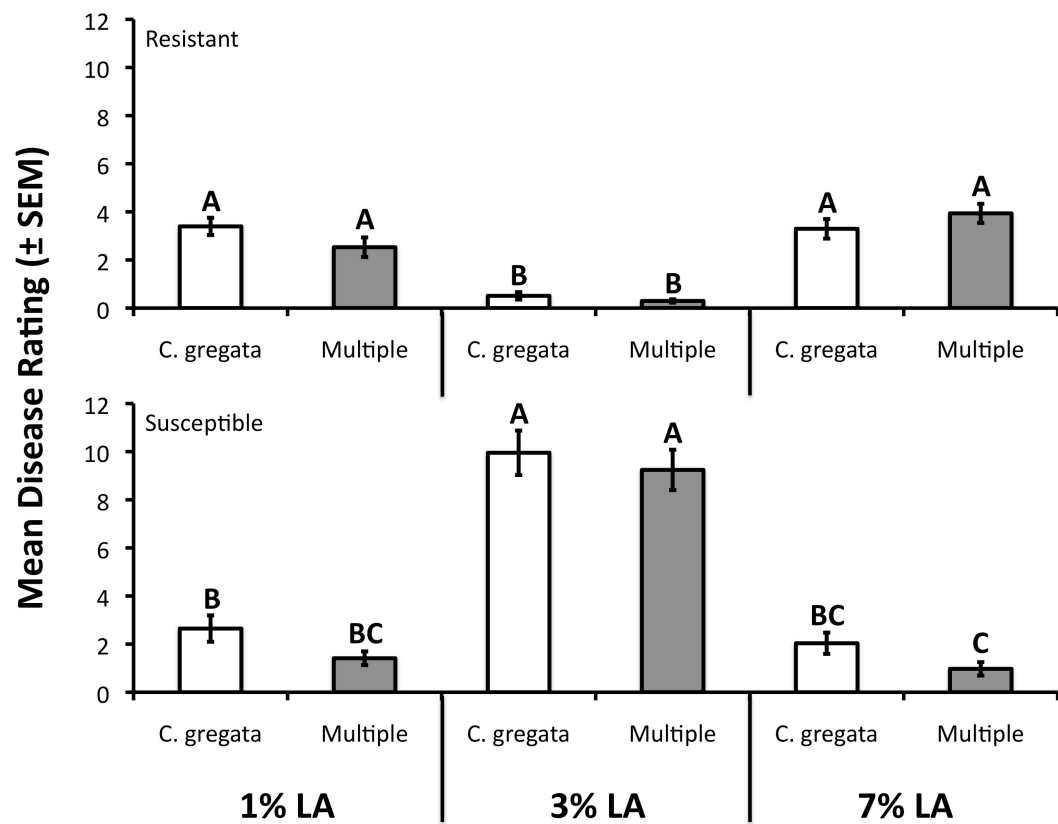
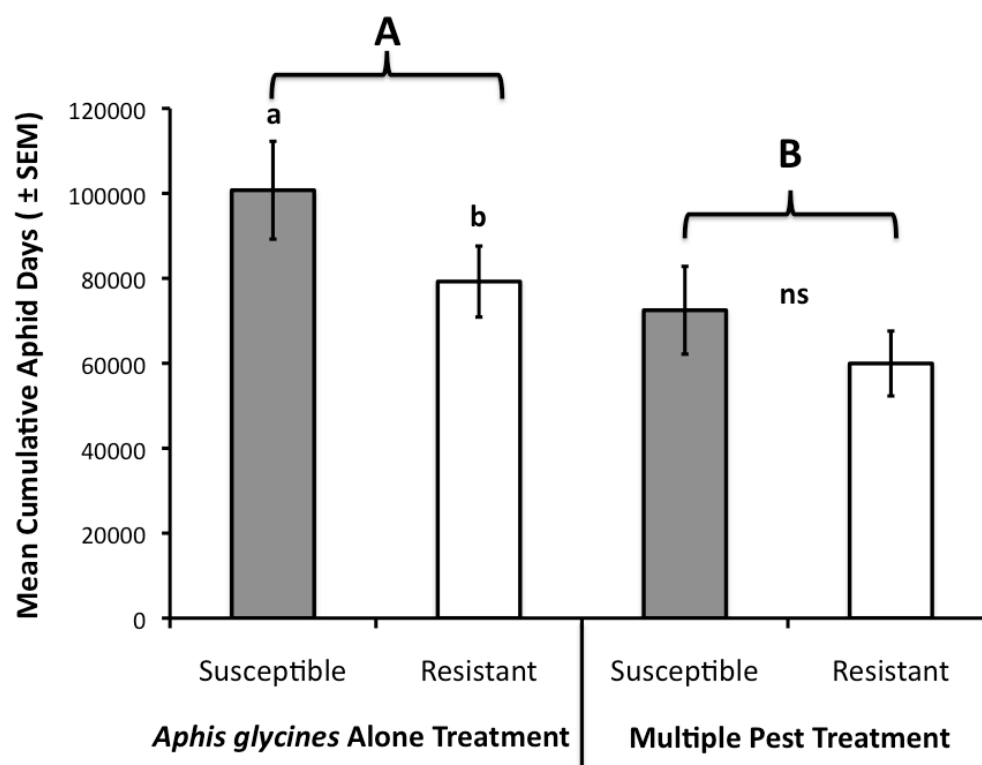


Figure 3.



Chapter Four

Measuring the yield and fatty-acid response of soybean cultivars with seed oil low in linolenic acid to multiple biotic stresses

A paper to be submitted to Crop Science

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Abstract

Linolenic acid is a precursor to components in the plant defense response to pests and pathogens. Commercial soybean cultivars with altered linolenic acid levels have been developed for their improved oil qualities. We examined how three common soybean pests, the soybean cyst nematode (SCN), brown stem rot (BSR) and the soybean aphid (SBA) affect the yield and seed linolenic acid levels of specialty low linolenic acid cultivars. In a micro-plot field experiment soybean cultivars with varying concentrations of linolenic acid in the seed (ultra-low or low) and resistance to SCN (susceptible or resistant) were grown at a research farm north of Ames, Iowa. Plots were kept free of pests (control) or artificially inoculated with a single pest (SCN, BSR or SBA) or the combination of all three pests (multiple pest). The use of resistant cultivars (SCN and BSR) or the use of an insecticide at an economic threshold (SBA) was analyzed to determine the efficacy of current management recommendations for low linolenic acid soybean production. Seed was collected at the end of the season to measure yield and grain composition. Fatty acid profiles of seeds were measured using GC-MS. Results indicate that all three pests alone or in combination are capable of reducing yield, but only BSR and SBA are capable of affecting seed linolenic acid levels. In all cases current management recommendations (resistant cultivars or economic thresholds) provided sufficient protection of both yield and grain composition indicating no changes are necessary for management of altered linolenic acid soybean varieties.

The development of integrated pest management strategies for agricultural production has been a goal of Midwestern soybean researchers, farmers and government agencies since the publication of Stern et al. (1959). Early research goals focus on the management of key soybean pests and pathogens in isolation and their impact on yield and aspects of grain quality (Adee 1994, Niblack et al 2005, Ragsdale et al. 2007, Beckendorf et al. 2008). Recent progress in plant breeding has also led to the development of soybean cultivars with altered seed traits that enhance their functionality and value. The development of improved integrated pest management strategies will require further knowledge on how current soybean pests interact with these new cultivars, specifically how they impact grain composition.

Soybean cultivars with modified fatty acid seed contents have been developed for improved end user traits, specifically functionality of the soybean oil (Fehr 2006). Cultivars with levels of linolenic acid (18:3) reduced from 8% to less than 3% were developed by chemical mutagenesis with ethyl methanesulfonate (EMS). The seed from these cultivars reduces or even eliminates the need for chemical hydrogenation. Chemical hydrogenation of soybean oil is used to increase the stability and shelf life of the oil. The process leads to the formation of saturated and *trans*-fatty acids, which have been linked to an increase in coronary heart disease (Department of HHS and USDA 2005, Chappel and Bilyeu 2007).

Farmers producing these specialty low linolenic acid soybean cultivars receive a premium above the price of commodity soybeans. This premium is

contingent upon the production of seed that is at or below a contracted level of seed linolenic acid content, as measured by percentage of total seed oil. Previous research has shown that environmental conditions such as temperature (Wolf et al. 1982) and rainfall (Dornbos and Mullen 1992) are capable of impacting fatty acid levels in soybean seed. Further studies have shown that environmental conditions that vary by year and location can impact grain composition, specifically linolenic acid content, in both commodity and low linolenic acid cultivars (Wilcox and Cavins 1992, Schnebly and Fehr 1993, Primomo et al. 2002, Oliva et al. 2006). Primomo et al. (2002) identified the need to understand the level to which weed, disease, and insect pressure can contribute to the significant variation in linolenic acid levels between years and locations observed in their study.

Linolenic acid has been identified as a precursor to key plant defense signaling compounds including jasmonic acid and green leaf volatiles (Shah 2005, Matsui 2006, Smith and Boyko 2007). Jasmonic acid has been identified as important in amplifying the initial defense response to pathogens and herbivores. Green leaf volatiles have also been identified as important defense signaling compounds within the plant and also as an important synomone used by predators to locate prey (Halitschke et al. 2008).

In the North Central United States several insect pests and many fungal and bacterial pathogens challenge soybean production. The most economically important insect pest is the soybean aphid (SBA), *Aphis glycines* Matsumura which was first reported in the region in 2000 (Heimpel and Shelly 2004). The SBA is a

phloem-feeding insect, which reproduces parthenogenetically on soybean in the summer. Soybean aphids are capable of reducing yields by as much as 40% (Ragsdale et al. 2007), as well as reducing seed oil content (Beckendorf et al. 2008). Current management relies heavily on insecticide applications applied at a previously determined economic threshold or according to a growth stage timed schedule (Myers et al. 2005, Ragsdale et al. 2007, Johnson et al. 2009). Recently, resistance genes to the SBA have been identified (Mensah et al. 2005, Hill et al. 2006b, a, Mian et al. 2008, Hill et al. 2009) and successfully integrated into Midwest-adapted cultivars (Mardorf et al. , Kim and Diers 2009). Micro-array studies of the resistant cultivar Dowling have shown lipoxygenase genes to be up regulated in response to SBA feeding (Li et al. 2008). The chief substrate of lipoxygenases is linolenic acid, which is converted to the plant hormone jasmonic acid (Shah 2005).

Soybean in the North Central United States is also attacked by a diverse group of pathogens including fungi, bacteria and viruses (Robertson and Nutter 2010). This group includes the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohoe and the fungal pathogen brown stem rot (BSR), *Cadophora gregata* Harrington and McNew. The SCN is the most economically important soybean pest in the United States, with damage estimated at over 1.5 billion dollars a year (Niblack et al. 2006). Current management relies on the planting of resistant cultivars in infested fields. Commercial SCN resistant cultivars incorporate a single source of resistance. Currently seven sources of resistance against the SCN have been identified in germplasm; three of which are available commercially, PI88788,

Peking and PI 437654 (also known as Hartwig or PUSCN 14) (Niblack 2005, Niblack et al. 2006). PI 88788 is the most commonly employed source of resistance, comprising the vast majority (>95%) of commercially available SCN resistant cultivars (Niblack et al. 2006). Recent studies have found a conserved gene expression mechanism between the resistance reactions of PI 88788 and Peking resistance sources, with a role for lipoxygenases in both sources (Klink et al. 2009, 2010a, 2010b).

Brown stem rot is a common fungal pathogen, present in 68-73 percent of soybean fields in Illinois, Iowa and Minnesota (Workneh et al. 1999). The BSR fungus infects the soybean plant through the root system and later colonizes the vascular and pith tissue of the plant impeding the movement of nutrients. BSR incidence and severity is positively impacted by SCN infection (Tabor et al. 2003, Tabor et al. 2006). Co-infection of SCN allows earlier colonization of the soybean roots by the fungus and possibly creates entry sites for the pathogen. Current BSR management relies on cultural control methods including crop rotation and tillage. Resistant cultivars are also available for control of BSR. When BSR and SCN occur within the same field, cultivars with resistance to both pathogens are recommended. The SCN resistance source PI88788 also displays some resistance to BSR in the absence of SCN infection (Hughes et al. 2004).

The goal of this experiment was to assess the impact of three common soybean pests, the soybean aphid, soybean cyst nematode and brown stem rot on the yield and seed linolenic acid content of altered low linolenic acid cultivars.

Further it was our goal to examine current integrated pest management tactics and their ability to protect yield and seed composition of altered low linolenic acid cultivars.

Materials and Methods

We conducted the following experiment from 2008 to 2009 at the Iowa State University Horticulture Research Station north of Ames, in Story Co. Iowa. A description of the experimental design is provided in Chapter 3 of this thesis. In short, six soybean cultivars varying in seed linolenic acid content were planted in replicated micro-plots (28 cm by 51 cm) kept 152 cm apart within six blocks. Six pest treatments were established in a complete factorial design with each treatment by cultivar present. This manuscript reports a sub-set of these treatment by cultivar combinations. For the analyses presented here, four cultivars with altered linolenic acid contents are included (Table 1).

Pest treatments are described in Chapters 2 and 3 of this thesis. In brief, six pest treatments were used consisting of three common Midwestern soybean pests, soybean cyst nematode (SCN), brown stem rot (BSR) and the soybean aphid (SBA). The first treatment was kept free of all pests and is referred to as the “control”. The second treatment consisted of SCN eggs applied in furrow as a 50mL water suspension and is referred to as the “SCN” treatment. The third treatment consisted of 40g of BSR infested sorghum seed and is referred to as the “BSR” treatment. Sorghum seed was mixed throughout the soil of the plot 24 hours prior to planting each year.

The fourth and fifth treatment consisted of SBA infestations. All SBA infestations were accomplished by applying five SBA to the upmost trifoliolate at the V3 to V4 growth stage (Fehr and Caviness 1977). The fourth treatment is referred to as the “SBA: unlimited” treatment. In this treatment SBA populations were allowed to grow on plants throughout the season, reaching densities well exceeding 1,000 aphids plant⁻¹. The fifth treatment, referred to as the “SBA: 250” treatment consisted of allowing SBA populations to reach the current economic threshold of 250 aphids plant⁻¹. Once plots assigned the SBA: 250 treatment obtained SBA populations exceeding the ET, these plots received a single application of λ -cyhalothrin (Warrior II with Zeon Technology) at the label rate for SBA. The final pest treatment referred to as “Multiple Pest” consisted of the combination of the SCN, BSR and SBA: unlimited treatments.

Planting occurred on 1 June in 2008 and 19 May in 2009 and 2010. Plots were planted with 22 seeds and thinned to 10 evenly spaced plants after soybean emergence. Plots were covered with cages at the VC to V1 growth stage and remained covered until harvest. Cages allowed for the manipulation of aphid population densities within plots. Cages consisted of PVC pipe frames measuring 1.1 m by 0.8 m by 0.8 m wide (height x length x width). White no-see-um mesh fabric (Quest Outfitters, Sarasota, FL) covered the frames preventing the movement of insects into or out of the cage.

Seed weight was used as a measure of yield. Plots were hand harvested at maturity. All seedpods were removed from each plant and seedpods of all plants of each plot were threshed in bulk. Seed was dried to a uniform 8% moisture and weighed in

grams using an electronic bench top scale. Linolenic acid concentration of seeds was analyzed as previously described by Hammond and Fehr (1984).

Statistical Analyses

Yield is reported as a ratio of the seed weight of a pest treatment plot divided by the seed weight of the corresponding control treatment plot. Seed linolenic acid concentration is reported as a ratio in the same manner as yield. Yield analyses were conducted separately for each pest (ie SCN, BSR and SBA) and the multiple pest treatment. The same general model was used for all analyses. A mixed effects model was used in which the fixed effects considered were block, linolenic acid level and nematode resistance. The effect of year was treated as a random variable. Significant effects in the model were then analyzed using least squares means analysis. This was done to test if the yield ratios of significant effects differed from the value of “1.0” indicating yields were significantly different from the control.

This general model was adapted for analysis of SBA treatments, due to multiple pest treatments in one analysis (SBA: unlimited, SBA: 250). Deviations from this model included the addition of the fixed effects of pest treatment and the two-way and three-way interactions of pest treatment with linolenic acid level and nematode resistance.

Ratios of seed linolenic acid content were analyzed using the same models and tests as outlined for yield analyses.

Results

Data were analyzed to address our hypotheses that (1) current IPM recommendations based on conventional soybean cultivars would also provide sufficient yield protection for specialty low linolenic acid soybean cultivars and that the (2) seed linolenic acid levels of these cultivars would be unaffected by the presence of soybean pests and pathogens.

Soybean Cyst Nematode

Yield analysis revealed the effects of linolenic acid level ($F = 0.3816$; $df = 1,26$; $P = 0.3816$) and nematode resistance ($F = 2.40$; $df = 1,26$; $P = 0.1338$) to be non-significant. The interaction of linolenic acid level and nematode resistance however was significant ($F = 6.67$; $df = 1,26$; $P = 0.0158$). Least squares means analysis was then performed by the interaction of linolenic acid and nematode resistance (Figure 1). Only the 3% SCN-S cultivar had a significantly reduced yield ($t = 0.2069$; $df = 26$; $P = 0.0118$).

Seed profile analysis revealed the effects of both linolenic acid content ($F = 2.13$; $df = 1,28$; $P = 0.1553$) and nematode resistance ($F = 0.35$; $df = 1,28$; $P = 0.5590$) to be non-significant. The interaction of linolenic acid level and nematode resistance was also non-significant ($F = 1.45$; $df = 1,28$; $P = 0.2392$). None of the seed linolenic acid ratios differed significantly from zero, indicating soybean cyst nematode infection was unable to impact seed linolenic acid concentration.

Brown Stem Rot

The effect of nematode resistance significantly affected yield in the brown stem rot treatment ($F = 9.59$; $df = 1,25$; $P = 0.0048$). The effect of linolenic acid level ($F = 0.39$; $df = 1,25$; $P = 0.5392$) and the interaction of nematode resistance and linolenic acid level ($F = 2.75$; $df = 1,25$; $P = 0.1098$) were non-significant. Least squares means analysis was then performed by the effect of nematode resistance (Figure 2). The yield of SCN-S cultivars was significantly reduced in the presence of BSR ($t = -2.96$; $df = 25$; $P = 0.0066$). The yield of SCN-R cultivars was not significantly reduced ($F = 1.22$; $df = 25$; $P = 0.2325$).

The effects of linolenic acid level ($F = 7.20$; $df = 1,28$; $P = 0.0121$) and nematode resistance ($F = 4.26$; $df = 1,28$; $P = 0.0484$) significantly affected seed linolenic acid ratios. The interaction of linolenic acid level and nematode resistance was marginally significant ($F = 3.26$; $df = 1,28$; $P = 0.0819$). Least squared means analysis was performed by cultivar (Figure 3), and indicated seed linolenic acid content was significantly elevated by brown stem rot infection in only the 3% SCN-S cultivar ($t = 3.22$; $df = 28$; $P = 0.0032$). Brown stem rot infection resulted in a 22% increase in seed linolenic acid concentration in the 3% SCN-S cultivar.

Soybean Aphid

The effect of pest treatment significantly affected yield ($F = 5.13$; $df = 1,45$; $P = 0.0284$). The effect of linolenic acid level was mildly significant ($F = 3.74$; $df = 1,12$; $P = 0.0769$). The remaining effect of nematode resistance ($F = 0.7489$; $df = 1,12$; $P = 0.7489$) and the interactions of linolenic acid level and nematode resistance ($F = 0.43$; $df = 1,12$; $P = 0.5265$), linolenic acid level and pest treatment ($F = 0.00$; $df = 1,12$; $P =$

0.9982), nematode resistance and pest treatment ($F = 0.05$; $df = 1,12$; $P = 0.8241$), and the three-way interaction of linolenic acid level, nematode resistance and pest treatment ($F = 0.22$; $df = 1,12$; $P = 0.6449$) were all non-significant. Least squares means analysis was performed by the effect of pest treatment (Figure 4). Yield was found to be significantly lowered in the SBA: unlimited treatment ($t = -0.2907$; $df = 44$; $P = 0.0016$) and unaffected in the SBA: 250 treatment ($t = -0.1047$; $df = 44$; $P = 0.2027$).

Analysis of seed linolenic acid ratios revealed the effects of pest treatment ($F = 0.24$; $df = 1,54$; $P = 0.6262$), linolenic acid level ($F = 2.85$; $df = 1,7$; $P = 0.1350$), and nematode resistance ($F = 0.14$; $df = 1,7$; $P = 0.7234$) to be non-significant. The two-way interactions between pest treatment, linolenic acid level and nematode resistance were all non-significant as well. The three-way interaction, however, was marginally significant ($F = 0.0740$; $df = 1,7$; $P = 0.0740$). Least squares mean analysis was performed by the three-way interaction and showed seed linolenic acid levels to be significantly elevated in the 1% SCN-S cultivar under the SBA: unlimited treatment ($t = 2.77$; $df = 7$; $P = 0.0278$) (Figure 5). The high aphid densities of the SBA: unlimited treatment resulted in a 9.5% increase in seed linolenic acid concentration in the 1% SCN-S cultivar.

Multiple Pest

Yield analysis revealed the effect of nematode resistance to be mildly significant ($F = 3.57$; $df = 1,27$; $P = 0.0698$). The effect of linolenic acid level ($F = 1.32$; $df = 1,27$; $P = 0.2613$) and the interaction of linolenic acid level and nematode resistance ($F = 2.86$; $df = 1,27$; $P = 0.1026$) were non-significant. Least squares means analysis was then performed by the effect of nematode resistance (Figure 6). Yield was significantly

reduced on both SCN-S ($t = -0.5151$; $df = 27$; $P < 0.0001$) and SCN-R ($t = -0.3501$; $df = 27$; $P < 0.0001$) cultivars. Susceptible cultivars however did experience mildly significant higher yield losses compared to resistant cultivars ($t = 1.89$; $df = 27$; $P = 0.0698$).

The effect of linolenic acid level had a significant impact on seed linolenic acid levels ($F = 5.12$; $df = 1,28$; $P = 0.0316$). The effect of nematode resistance was marginally significant ($F = 4.07$; $df = 1,28$; $P = 0.0533$). The interaction between linolenic acid level and nematode resistance was non-significant ($F = 0.61$; $df = 1,28$; $P = 0.4408$). Least squares means analysis was performed by cultivar (Figure 7). Seed linolenic acid level was significantly elevated in the 1% SCN-S cultivar ($t = 3.86$; $df = 28$; $P = 0.0006$). Seed linolenic acid concentration increased by 14.5% in the 1% SCN-S cultivar when challenged by multiple pests.

Discussion

The crop value of altered low seed linolenic acid cultivars is dependent upon both the yield or amount harvested and the linolenic acid content of the seed harvested. Previous work has identified temperature during pod fill as capable of impacting seed oil composition. Environmental and cultural practices capable of influencing temperature during pod fill include planting date, year, and location (Wilcox and Cavins 1992, Schnebly and Fehr 1993, Primomo et al. 2002, Oliva et al. 2006). Primomo et al. (2002) found the effect of year to have a stronger impact than location and identified the need to understand how insect or other pest pressure may contribute to the variability in oil

composition from year to year. In our study, both yield and seed linolenic acid content was impacted by pest treatment and cultivar selection. This result highlights the need for IPM tactics to protect the value of this specialty crop. Our study evaluated both the potential for crop pests to affect yield and seed linolenic acid content and the efficacy of current IPM recommendations to protect yield and seed composition.

Yield data analyses indicated that all three pests used in this experiment were capable of significantly reducing yields of altered seed linolenic acid cultivars. The planting of nematode resistant cultivars carrying the PI 88788 resistance provided sufficient yield protection in both the SCN and BSR treatments (Figures 1 and 2). The application of insecticides according to the current economic threshold of 250 aphids plant⁻¹ also led to sufficient yield protection in the SBA: 250 treatment (Figure 4).

Analyses of seed linolenic acid contents revealed both BSR and SBA capable of increasing seed linolenic acid contents (Figures 3 and 5), while SCN was not. It should be noted, however that the 2008 and 2009 growing seasons in Iowa had unusually high rainfalls and 2009 was also unusually cool. Cool weather and adequate rainfall can mitigate the impact of SCN on the soybean plant. It is possible that for SCN our results could be very different if repeated in a season with hot, dry weather, conditions that magnify the impact of SCN on soybean.

In the case of BSR, significantly elevated linolenic acid content was only observed on the 3% SCN-S cultivar (Figure 3), which also suffered the most significant yield reduction (Figure 2). Cultivars containing the PI 88788 SCN resistance did not have elevated seed linolenic acid contents. These results indicate the effectiveness of

protecting seed composition by reducing the severity of BSR disease, which can be accomplished through the planting of resistant cultivars. For cultivars exposed to SBA, elevated seed linolenic acid contents were only observed for the 1% SCN-S cultivar exposed to the high aphid densities of the SBA: unlimited treatment (Figure 5). This result indicated that the use of an ET is effective in protecting the seed composition of altered low linolenic acid cultivars.

The results of this experiment taken as a whole indicate that for altered low linolenic acid cultivars, SCN is capable of reducing yield and BSR and SBA are capable of reducing both yield and seed linolenic contents. The largest impact we measured on seed linolenic acid contents was an increase of 22%, this is a relatively small increase not likely to result in a reduction in the value of the crop. Also our study found that current IPM recommendations, which were developed for conventional soybean production, provide sufficient yield and seed composition protection for altered low linolenic acid cultivars. Indicating that current recommended management practices should be sufficient for low linolenic acid soybean production.

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Table 1. Low linolenic acid cultivars planted in 2008 and 2009

Cultivar	LA ^a Content	Nematode Resistance ^b
IA 3018 ^c	3%	Susceptible
AG 2821 V	3%	PI 88788
IA 3041	1%	Susceptible
IA 3028	1%	PI 88788

^a Linolenic acid seed content as measured as percentage of whole oil content

^b Resistance to soybean cyst nematode

^c IA 3018 not available in 2008, but added to experiment for 2009

Figure Captions

Figure 1. Exposure to soybean cyst nematode (SCN) resulted in a significant yield reduction. Yield was significantly reduced on the SCN-susceptible 3% linolenic acid ($t = 0.2069$; $df = 26$; $P = 0.0118$). Mean yield ratios of all four cultivars in the SCN alone treatment are displayed. The yield ratio was calculated as the yield of the SCN treatment plot divided by the yield of the control plot. Asterisks denote yield ratios, which are significantly different from the value of 1.0.

Figure 2. Mean yield ratios of soybean cyst nematode (SCN) susceptible and resistant cultivars exposed to the brown stem rot (BSR) alone treatment. The yield ratio was calculated as the yield of the BSR treatment plot divided by the yield of the control plot. Different letters represent significant differences among means. Yield was significantly greater on SCN-resistant cultivars than SCN-susceptible cultivars ($F = 9.59$; $df = 1,25$; $P = 0.0048$). Asterisks denote yield ratios, which are significantly different from the value of 1.0. Indicating exposure to BSR resulted in a significant yield reduction. Yield was significantly reduced on SCN-susceptible cultivars ($t = -2.96$; $df = 25$; $P = 0.0066$).

Figure 3. Mean seed linolenic acid content ratios of all four cultivars in the brown stem rot (BSR) alone treatment. Ratios were calculated as the seed linolenic acid content of the BSR treatment plot divided by the seed linolenic acid content of the corresponding control plot. Analyses revealed seed linolenic acid content was significantly affected by linolenic acid level ($F = 7.20$; $df = 1,28$; $P = 0.0121$), soybean cyst nematode resistance ($F = 4.26$; $df = 1,28$; $P = 0.0484$). The interaction of linolenic

acid level and nematode resistance was also marginally significant ($F = 3.26$; $df = 1,28$; $P = 0.0819$). Different letters represent significant differences among means. The ratio of seed linolenic acid level was significantly greater on the 3% SCN-susceptible cultivar compared to both 1% cultivars. Asterisks denote ratios, which are significantly different from the value of 1.0. Indicating exposure to BSR resulted in a significant increase in seed linolenic acid content. Seed linolenic acid content was significantly elevated on the 3% SCN-susceptible cultivar ($t = 3.22$; $df = 28$; $P = 0.0032$).

Figure 4. Mean yield ratios of cultivars (1% and 3% LA) exposed to treatments of two different densities of soybean aphid (SBA); the SBA: 250 treatment and the SBA: unlimited treatment. Means are calculated over all cultivars exposed to a given treatment. The yield ratio was calculated as the yield of the SBA treatment plot divided by the yield of the corresponding control plot. Different letters represent significant differences among means. Yield was significantly greater in the SBA: 250 treatment compared to the SBA: unlimited treatment ($F = 5.13$; $df = 1,45$; $P = 0.0284$). Asterisks denote yield ratios, which are significantly different from the value of 1.0. Indicating exposure to that treatment resulted in a significant yield reduction. Yield was significantly reduced on cultivars exposed to the SBA: unlimited treatment ($t = -0.2907$; $df = 44$; $P = 0.0016$).

Figure 5. Mean seed linolenic acid ratios of cultivars exposed to treatments of two different densities of soybean aphid (SBA); the SBA: 250 treatment and the SBA: unlimited treatment. The seed linolenic acid ratio was calculated as the seed

linolenic acid content of the SBA treatment plot divided by the seed linolenic acid content of the corresponding control plot. Different letters represent significant differences among means. Asterisks denote seed linolenic acid ratios, which are significantly different from the value of 1.0. Indicating exposure to that treatment resulted in a significant increase in seed linolenic acid content. Seed linolenic acid content was significantly elevated on only the 1% soybean cyst nematode susceptible cultivar exposed to the SBA: unlimited treatment ($t = 2.77$; $df = 7$; $P = 0.0278$).

Figure 6. Mean yield ratios of soybean bean cyst nematode (SCN) susceptible and resistant cultivars in the multiple pest treatment. The multiple pest treatment consisted of exposing plots to the combination of the SCN alone, brown stem rot (BSR) alone, and soybean aphid (SBA) unlimited treatments. The yield ratio was calculated as the yield of the multiple pest treatment plot divided by the yield of the control plot. Asterisks denote yield ratios, which are significantly different from the value of 1.0. Indicating exposure to multiple pests resulted in a significant yield reduction. Yield was significantly reduced on both SCN-susceptible ($t = -0.5151$; $df = 27$; $P < 0.0001$) and SCN-resistant ($t = -0.3501$; $df = 27$; $P < 0.0001$) cultivars.

Figure 7. Mean seed linolenic acid content ratios of all four cultivars in the multiple pest treatment. The multiple pest treatment consisted of exposing plots to the combination of the SCN alone, brown stem rot (BSR) alone, and soybean aphid (SBA) unlimited treatments. Ratios were calculated as the seed linolenic acid content of the multiple pest treatment plot divided by the seed linolenic acid content

of the corresponding control plot. Seed linolenic acid content was significantly affected by linolenic acid level ($F = 5.12$; $df = 1,28$; $P = 0.0316$) and soybean cyst nematode resistance ($F = 4.07$; $df = 1,28$; $P = 0.0533$). Different letters represent significant differences among means. The ratio of seed linolenic acid level was significantly greater on the 1% SCN-susceptible cultivar compared to the 3% SCN-resistant cultivar. Asterisks denote ratios, which are significantly different from the value of 1.0. Indicating exposure to multiple pests resulted in a significant increase in seed linolenic acid content. Seed linolenic acid content was significantly elevated on the 1% SCN-susceptible cultivar ($t = 3.86$; $df = 28$; $P = 0.0006$).

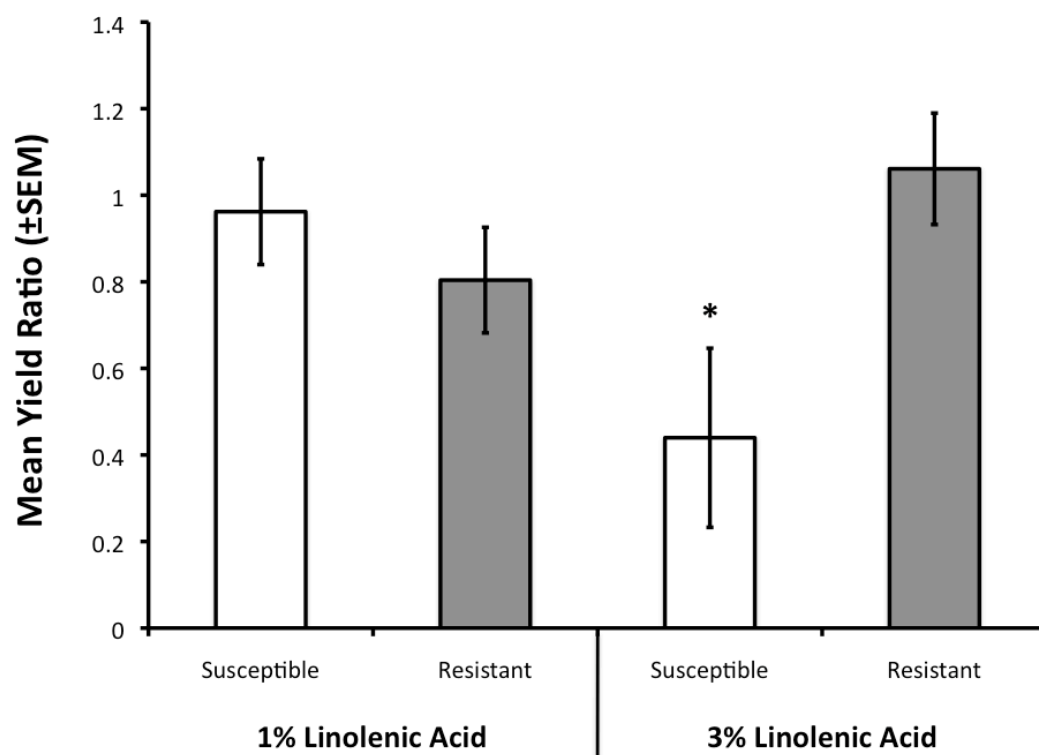
Figure 1.

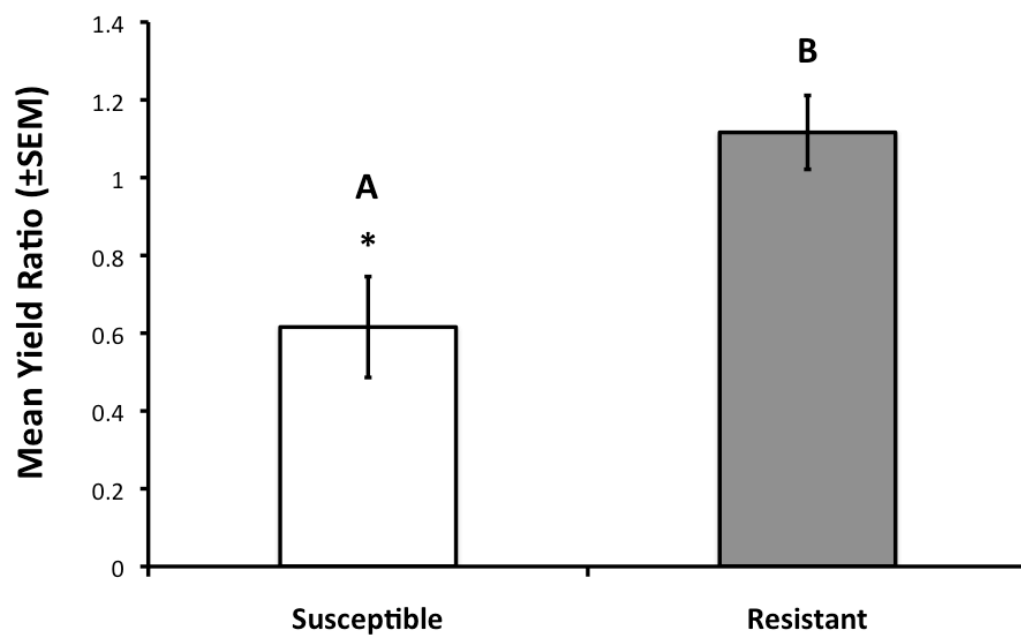
Figure 2.

Figure 3.

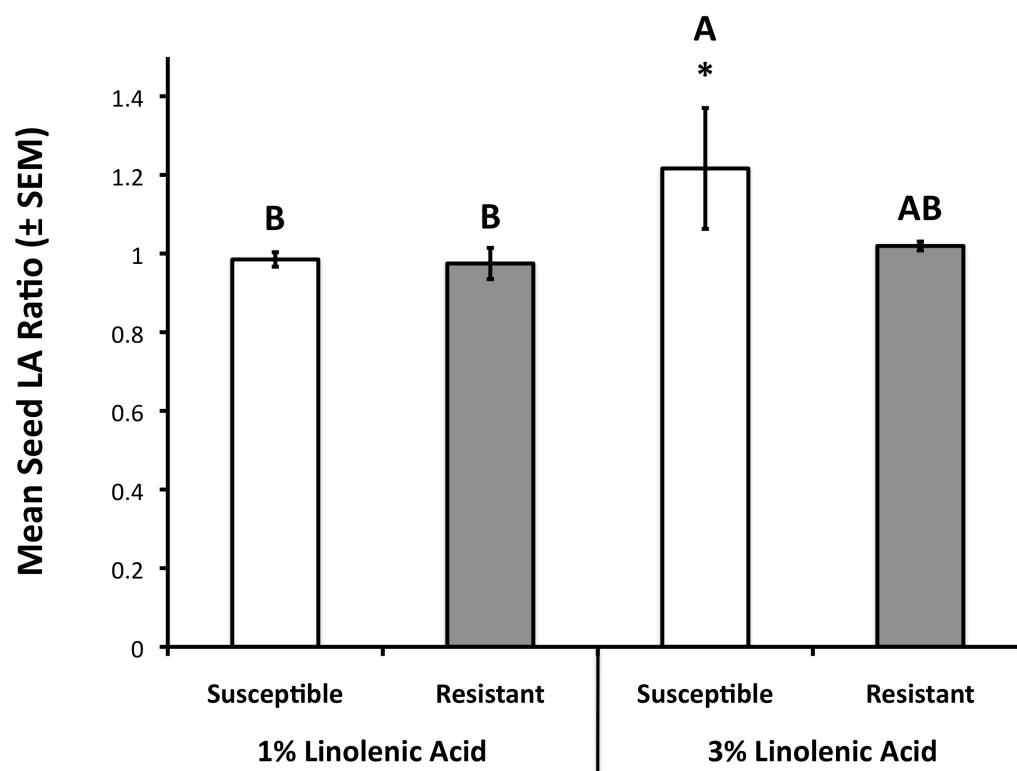


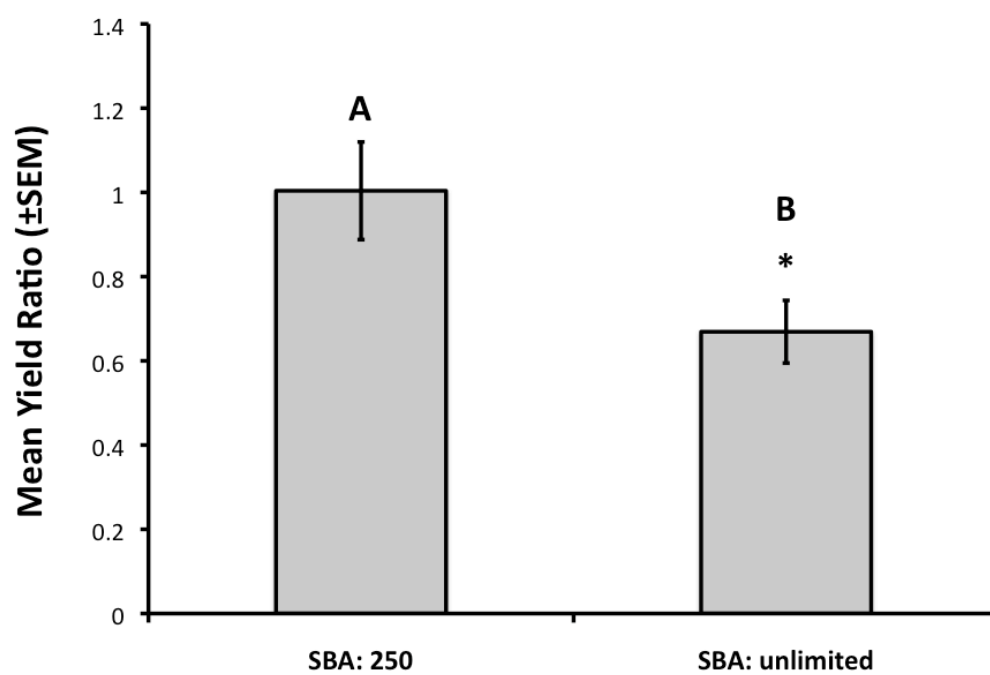
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Figure 5.

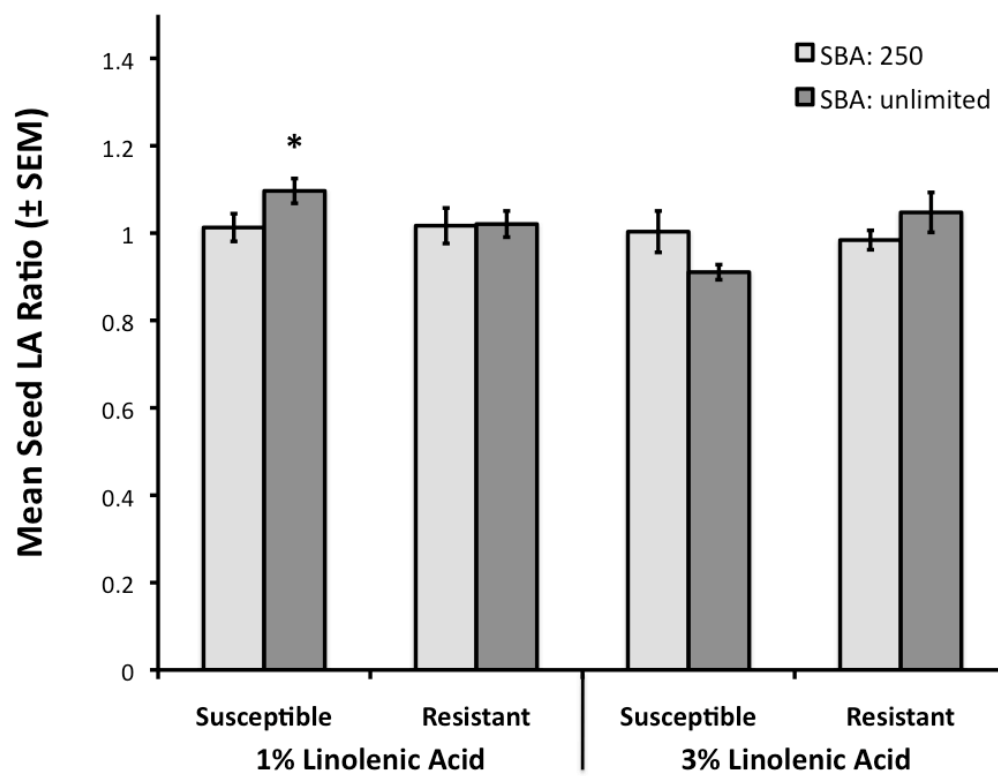


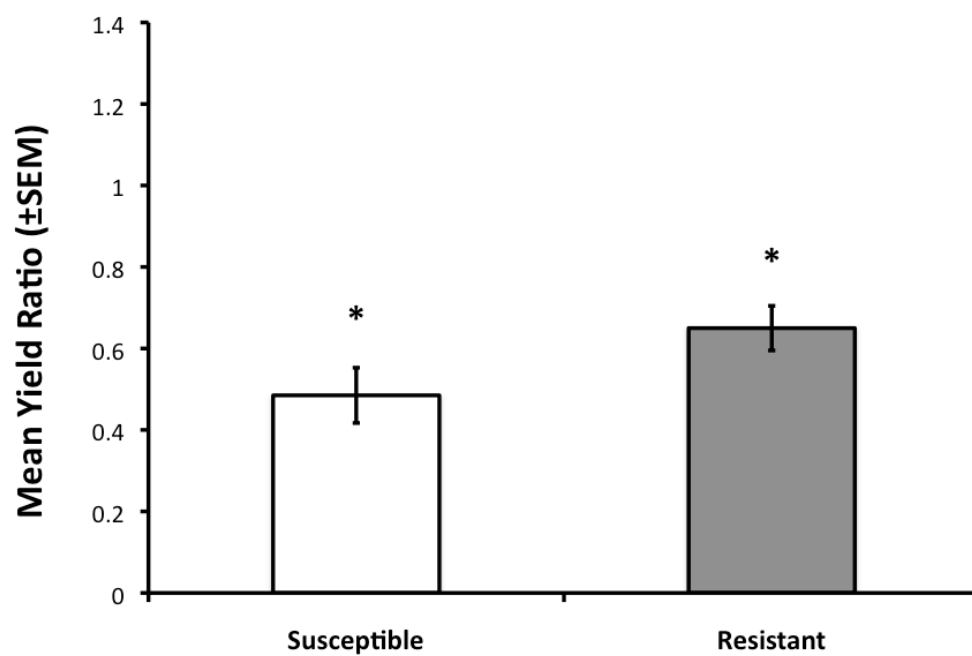
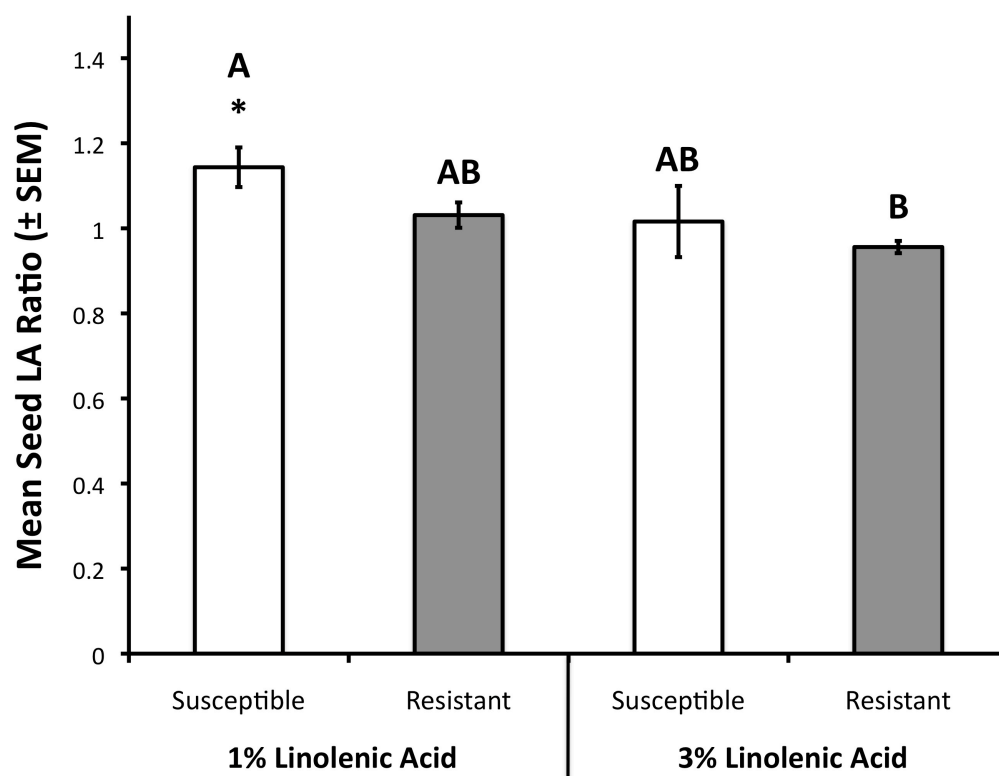
Figure 6.

Figure 7.



Chapter Five

Impact of altered linolenic acid levels on bean leaf beetle (Coleoptera: Chrysomelidae) preference for soybean volatiles

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Abstract

Linolenic acid is a precursor to volatile defense compounds in soybean. We examined the effect of altered fatty acid synthesis pathways on the volatile emissions of soybean plants. We used a dual-choice olfactometer to measure the olfactory preference of female bean leaf beetles (BLBs) *Cerotoma trifurcata* for soybean cultivars varying in seed linolenic acid content. Three soybean cultivars with varying contents of linolenic acid (ultra-low, low, conventional) were compared. Plants of each cultivar were tested at early growth stages, when BLBs colonize soybean fields in Iowa. Visual cues were eliminated in the olfactometer to focus plant selection to olfactory cues. Preference of BLBs was measured for wounded and unwounded plants of these cultivars. Wounded plants were exposed to two female BLBs for 24 hours immediately before being placed in the olfactometer. A preference was measured for wounded conventional plants compared to unwounded conventional plants. No preference was observed between wounded and unwounded plants of the ultra-low linolenic acid cultivar. In two follow up experiments BLBs showed no preference between unwounded plants of each cultivar. A preference was observed for volatile blends of wounded plants of both the conventional and low linolenic cultivars compared to the wounded ultra-low linolenic cultivar. The results of these experiments indicate a BLB perceivable change in the induced volatile profile of ultra-low linolenic acid soybean cultivars that leads to a loss of preference for the induced volatile blend of wounded plants of this cultivar.

The bean leaf beetle (BLB), *Cerotoma trifurcata* Forster is a sporadic pest of soybean in Iowa causing yield losses through defoliation, pod feeding and virus transmission (Krell 2002, Krell et al. 2003, 2004, Smelser and Pedigo 1992a,b). In Iowa three generations of BLBs can be found in a single year (Smelser and Pedigo 1991). The overwintering generation of BLBs is the primary population responsible for colonizing soybean fields in the spring. The cues used by these beetles to locate and select soybean fields for oviposition are currently unknown.

The overwintering generation (F_0) of BLBs emerges primarily from woodlands and soybean fields (Lam et al. 2002). The overwintering generation first moves into alfalfa fields where they can be found from late April to early June. In May, BLB populations in alfalfa begin to decrease as they move from alfalfa to soybean fields to feed on the newly emerging soybean plants (Smelser and Pedigo 1991). The overwintering generation oviposits in soybean and give rise to the F_1 generation. The F_1 generation emerges in soybean and populations peak in mid-July to early August. The F_2 generation emerges in August and feeds on soybean leaf tissue and pods until plant senescence. Upon soybean senescence, BLBs leave soybean fields in search of overwintering habitats (Smelser and Pedigo 1991).

Early and mid-season management of the BLB can be critical for limiting the establishment and spread of bean pod mottle virus (BPMV) and the build-up of high late season BLB populations. Current management options for the BLB

and BPMV include both seed and foliar insecticide treatments and delayed planting dates. These tactics have shown some effectiveness in managing both BLBs and BPMV (Pedigo and Zeiss 1996, Krell et al. 2004, 2005, Bradshaw et al. 2008).

In soybean, BLB feeding is known to upregulate lipoxygenase activity leading to induced resistance to further BLB feeding (Felton et al. 1994 Srinivas et al. 2001). In BLBs, little is known about olfactory cues used for host orientation or the effect of herbivore induced volatiles on host plant preference. In soybean, Japanese beetles, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) are preferentially attracted to wounded foliage compared to unwounded foliage, and green leaf volatiles have been hypothesized to play a role in increasing attraction (O'Neill et al. 2010).

Herbivorous insects in the family Chrysomelidae, commonly referred to as leaf beetles, rely on plant odors as well as visual cues for locating suitable host plants. Qualitative and quantitative differences in volatile blends exist between plant species, within plant species and within time. These differences can be exploited by Chrysomelid herbivores to locate a suitable host plant. Plants, however, are not without defense as volatile profiles can be altered following challenge by an herbivore or pathogen. These changes can both deter or attract further herbivore colonization. The outcomes of these interactions are specific to the plant, challenging organism and searching herbivore (see review in Fernandez and Hilker 2007).

Linolenic acid is an important precursor to defensive compounds produced by plants against herbivorous insects (Walling 2000). One set of plant defensive compounds; the green leaf volatiles are derived from linolenic acid through the action of the enzyme hydrogen peroxide lyase, which is part of the lipoxygenase pathway (Matsui 2006). Green leaf volatiles are volatile organic compounds (VOCs) that are constitutively produced by plants. Qualitative and quantitative changes in their emission are important in herbivore attraction and plant defense through herbivore repulsion and predator attraction.

Green leaf volatiles are responsible for attracting the Colorado potato beetle, *Leptinotarsa decmlineata* Say (Coleoptera: Chrysomelidae) to potato (WeiBbecker et al. 1997). In this plant, green leaf volatiles are produced prior to insect damage, and their emission is highly up regulated by Colorado potato beetle feeding (Gosset et al. 2008). Colorado potato beetles are attracted to potato plant volatiles in olfactometer experiments. This attraction is significantly heightened when insects are presented with volatiles of plants previously fed on by Colorado potato beetles (Bolter et al. 1996, Landolt et al. 1999).

Oil derived from soybean seed accounts for over 80% of all vegetable oil consumed in the United States (DiRenzo et al. 2006). The oil produced from commodity soybean seeds is oxidatively unstable, due primarily to the three double bonds in linolenic acid. Manufacturers chemically hydrogenate the oil to stabilize it. This process increases the shelf life of the oil and also gives it a more

desirable texture. The process of hydrogenization leads to the production of saturated fats and trans fats (Chappel and Bilyeu 2007). As of January 2006, the Food and Drug Administration requires all food manufacturers to provide trans fatty acid content information on labels. This regulation came as a response to research that trans fatty acids have negative effects on blood cholesterol levels, leading to increased risks for coronary heart disease (Department of HHS and USDA 2005). Soybean cultivars with altered seed fatty acid profiles have been desired for improved functioning and nutritional qualities. Soybean oil derived from seed with reduced linolenic acid content is able to reduce or eliminate the need for chemical hydrogenation.

Low linolenic acid soybean lines were first developed at Iowa State in the 1970s using chemical mutagenesis (Hammond and Fehr 1984a,b). Low-linolenic acid cultivars have reduced seed linolenic acid content ranging from 3.5-1% compared to traditional cultivars with contents around 8%. The soybean genome has three *FAD3* genes; which are responsible for the conversion of linoleate to linolenate (Bilyeu et al. 2003) in the endoplasmic reticulum. Lines with reduced seed linolenic acid levels have disrupted function of one or more *FAD3* genes (Bilyeu et al. 2003, Bilyeu et al. 2005, Anai et al. 2005, Chapell and Bilyeu 2006,2007). While these mutations dramatically affect the level of linolenic acid in seeds, leaf linolenic acid is not necessarily affected due to the presence of an alternative pathway for unsaturated fatty acids production in the

chloroplast. Low linolenic acid soybean lines have been grown commercially since 1994 (Fehr 2007).

Given the role linolenic acid derived VOCs have on the attraction of chrysomelid herbivores in other systems; our objective was to characterize what effect cultivars with modified fatty acid synthesis pathways would have on BLB attraction. We hypothesized that low-linolenic acid cultivars would produce fewer green leaf volatiles and be less attractive to BLBs. We further hypothesized that this effect would vary between undamaged and damaged soybean plants.

Materials and Methods

We conducted three experiments to characterize the response of BLBs to olfactory cues from soybean cultivars with varying levels of seed linolenic acid (LA) content. A 180° dual-choice olfactometer was used for all experiments (Figure 1). The olfactometer was constructed of two 10.3 cm x 20.6 cm (diameter x depth) plastic satellite containers connected to a single central 9.8 cm x 6.5 cm plastic container (Pioneer Plastics Dixon, KY). The single central container was blackened out with a layer of black electrical tape (3M Corporation, St. Paul, MN) around the entire outside surface. The central chamber was connected to the satellite chambers by a 2.5 cm diameter piece of tygon tubing measuring 8cm in length. Tygon tubing was inserted into the central chamber and satellite chambers with \approx 2 cm overhang on the inside of

each chamber. The tubing was then secured in place with strips of electrical tape placed on the outside of each chamber/tube connection. A vacuum pump (Model 22D1180-201-1003, Gast Manufacturing, Benton Harbor, MI) was attached to the top of the central chamber and was used to pull air from the satellite chambers into the central chamber. A 0.5 cm diameter hole, 3.5 cm from the base of each satellite chamber and opposite from the tube connecting the satellite and central chambers facilitated air movement within the olfactometer. The hole was covered with an activated charcoal filter to prevent outside VOCs from entering the olfactometer.

Bean Leaf Beetles

Bean leaf beetles were collected in 2010 from commercial soybean fields in Boone County, Iowa. Bean leaf beetles came from a single field selected for having a high BLB population during the F₁ generation. Two generations were sampled, the F₁ and F₂. For each generation, BLBs were collected from the field and maintained in the lab on soybean leaf tissue collected from the same field in which the BLBs were collected. Supplemental water was also provided via moistened cotton wicks.

Bean leaf beetles were kept in the laboratory for a period no longer than three weeks before being tested in an olfactometer. Bean leaf beetles were starved (water was still provided) for 24 hours immediately prior to being placed in an olfactometer.

Plant Cultivars

Three plant cultivars differing in seed LA content grown commercially in Iowa were selected. The three cultivars used included an ultra-low LA variety, IA 3027 (1% LA), a low LA cultivar, AG 2821 V (3% LA) and a commodity cultivar, DK 27-52 (7% LA). Seeds of each cultivar were planted in clear plastic cups 9cm in diameter and 7cm in height (Solo Cup Corporation Highland Park, IL). Plants were grown to the vegetative stages (early V1 to late V2 stage, Fehr and Caviness 1977) before being used in experiments. These early vegetative stages are typical for the time of year when BLBs colonize soybean fields within Iowa (Smelser and Pedigo 1991). Plants were grown in a greenhouse maintained at $30 \pm 5^{\circ}\text{C}$ with a 14:10 (L:D) photoperiod supplemented with metal halide illumination.

Bean leaf beetles were kept from feeding on plants during the experiments by a white no-see-um mesh (Quest Outfitters, Sarasota, FL) placed over the plants and secured with a rubber band. Plants were randomly assigned to each arm of the olfactometer using a random number generator.

Ten starved BLBs were placed in petri dishes. A single petri dish was placed in the bottom of the central chamber of each olfactometer. The lid of each petri dish was then removed and the top of the central chamber was secured shut with a strand of electrical tape placed completely around. Olfactometers were kept at a room temperature set at $24 \pm 2^{\circ}\text{C}$ with a 12:12 (L:D) photoperiod.

Bean leaf beetles were given 24 hours to select an arm of the olfactometer. Visual non-destructive counts were performed at 2, 4 and 8 hours after initiation of the experiment. The location of all visible BLBs was recorded. At 24 hours after initiation each olfactometer was unhooked from its vacuum pump and the location of each BLB was determined through a destructive count in which the olfactometer was completely disassembled.

In all experiments, after completion of the final count of BLBs in the satellite and central chamber, the height, leaf area and leaf area removed of each soybean plant was measured. Height was determined as the distance between the soil line and the apical meristem of the plant. Leaf area and leaf area removed were determined by removing the unifoliate and trifoliate leaves of each plant at the point of attachment to the stem. Digital images of each leaf were made using the greyscale photograph setting of a desktop scanner (Epson 2450, Epson America, Inc., Long Beach, CA). The leaf area and leaf area removed were estimated from these digital files using the Image J software program according to O'Neal et al. (2002).

Experiment 1. Constitutive vs. Induced Volatile Profiles

We tested the preference of BLBs for induced volatiles by comparing the preference of beetles for wounded and unwounded plants of the same cultivar. We also assessed the ability of altered linolenic acid content plants to activate induced volatiles. The 7% LA and 1% LA cultivars were used for this experiment. This created two treatments for Experiment 1. The first was the

comparison of a 7% LA unwounded plant to a 7% LA wounded plant. The second was the comparison of a 1% LA unwounded plant to a 1% LA wounded plant.

Wounding of plants was accomplished by placing two female BLBs on each plant for 24 hours immediately prior to an experimental run. Beetles were confined to each plant's plastic cup using the same no-see-um nets previously described. After 24 hours nets were taken off the plastic cups and all female beetles were removed from the plants. Nets were then placed back over the plastic cups and plants were randomly assigned to each arm of the olfactometer. At the conclusion of each experiment after beetles were counted a measurement of plant height was taken. Measurements of leaf area and leaf area defoliated were also taken using Image J.

Experiment 1 was replicated with BLBs from different generations over the course of multiple days. In a single day, nine separate olfactometers were utilized. Olfactometers were divided into groups of three; each group was linked to a single vacuum pump with airflow divided evenly by a gang valve. A completely randomized design was used for Experiment 1.

Differences in BLB availability led to uneven replication between generations. Three days and two days of testing were conducted for the F_1 and F_2 generations respectively. On the final day of testing for the F_1 generation; one olfactometer was left unused to achieve equal replication among treatments. In total there were 43 replications across five days and two generations of BLBs.

Differences between 1% LA and 7% LA cultivars in preference for induced versus constitutive volatile blends in Experiment 1 led to the formation of two alternate hypotheses. First, the 1% LA cultivar constitutively produces an induced volatile blend, ie the constitutive volatile blend of the 1% LA cultivar is comparable to the induced volatile blend of the 7% LA cultivar. To test this hypothesis we conducted a second experiment analyzing BLB preference for the volatile blend of unwounded plants differing in linolenic acid content. Our second hypothesis was, the 1% LA cultivar does not mount a volatile response to BLB feeding. To test this hypothesis we conducted a third experiment analyzing BLB preference for the induced volatile blends of cultivars differing in linolenic acid content.

Experiment 2. Constitutive Volatile Profiles

Unwounded soybean plants were used to test if the preference of BLBs to the constitutive volatile profiles varied by soybean cultivar. Bean leaf beetles were exposed to two different cultivars for a total of three treatments (1% LA and 3% LA, 1% LA and 7% LA, and 3% LA and 7% LA). These experiments were replicated with BLBs from different generations over the course of multiple days. In a single day, nine separate olfactometers were utilized. Olfactometers were divided into blocks of three; each block consisting of three olfactometers linked to a single vacuum pump with air flow divided evenly by a gang valve. Within a day, treatments were assigned to olfactometers in a randomized complete block design.

At the conclusion of each day following the final destructive BLB counts the additional measurements of plant height and leaf area were taken as described previously. Five and three days were completed with the F₁ and F₂ generations respectively. This sampling accounted for 72 reps across eight days (9 replications day⁻¹) and two generations.

Experiment 3. Induced Volatile Profiles

Wounded soybean plants were used to examine the preference of BLBs for the induced volatile profiles of cultivars differing in seed LA content. Plant wounding was accomplished using two female BLBs and the same methods from Experiment 1. All other experimental protocols from Experiments 2 were used again including the randomized complete block design for each day. The measurements of plant height and leaf area were taken. The additional measurement of leaf area removed was taken using the same methods as previously described.

Again differences in availability of BLBs led to uneven replication among generations. Five days and three days of testing were performed for the F₁ and F₂ generations respectively. In total 72 replications occurred over eight days and two generations of BLBs.

Statistical Analyses

Odds ratios were calculated to analyze BLB preference. Odds ratios were calculated and log transformed according to the following equations:

$$\text{Log odds} = \log (\text{Cultivar a} / \text{Cultivar b})$$

Where “cultivar a” equals the number of BLBs present on plant “a” at 24 hours and “cultivar b” represents the number of BLBs on the opposing plant “b” at 24 hours. This formula was amended as follows;

If “Cultivar a” equals zero then,

$$\text{Log odds} = \log (1/[2 (\text{Cultivar a} + \text{Cultivar b})])$$

if “Cultivar b” equals zero then,

$$\text{Log odds} = \log (1-1/2 [\text{Cultivar a} + \text{Cultivar b}]).$$

Log odds ratios were then used to analyze beetle preference using PROC MIXED (SAS Institute 2001). Statistical analyses were conducted separately for each experiment.

Data from all three experiments were analyzed using a similar model for all experiments. The basic model included the fixed terms generation, day nested within generation, treatment and the interaction of treatment and generation. Additional terms included the terms leaf area ratio, height ratio and defoliation ratio. Leaf area ratio was calculated as

$$\text{Leaf area ratio} = \text{Leaf area Cultivar a} / \text{Leaf area Cultivar b}.$$

The terms height ratio and defoliation ratio were calculated in the same manner using plant height and leaf area removed, respectively. Additional terms were added to the basic model as the fixed effects of height ratio and leaf area ratio for Experiment 1. Also for Experiment 1, the leaf area removed of the wounded plant was incorporated into the model as the fixed effect of leaf area removed.

The model for Experiment 2 included the additional fixed effects of the log of the

height ratio and leaf area ratio. The fixed effects of height ratio, defoliation ratio and leaf area ratio were included in the model for Experiment 3. Least squares means analysis was used as before to measure differences in BLB preference.

Results

We examined the preference of two generations of BLBs. In total 1,880 female BLBs were used to test our hypotheses.

Experiment 1

Analyses revealed all main effects to be non-significant; generation ($F = 0.15$; $df = 1,33$; $P = 0.7039$), day(generation) ($F = 1.35$; $df = 3,33$; $P = 0.2740$), treatment ($F = 1.41$; $df = 1,33$; $P = 0.2436$), height ratio ($F = 0.07$; $df = 1,33$; $P = 0.7990$), leaf area ratio ($F = 0.05$; $df = 1,33$; $P = 0.8298$), leaf area removed ($F = 0.69$; $df = 1,33$; $P = 0.4126$) and generation*treatment ($F = 0.07$; $df = 1,33$; $P = 0.7918$). Least squared means analysis revealed no effect of wounding on preference for the 1% LA cultivar ($t = -0.08$; $df = 33$; $P = 0.9363$). A mildly significant preference for wounded plants compared to unwounded plants was found in the 7% LA cultivar ($t = -1.78$; $df = 33$; $P = 0.0839$). For the 7% LA cultivar, BLBs were 1.48 times more likely to select the wounded plant compared to the unwounded plant (Figure 2).

Experiment 2

None of the fixed effects of generation ($F = 0.25$; $df = 1,58$; $P = 0.6220$), treatment ($F = 0.77$; $df = 2,58$; $P = 0.4693$), day ($F = 0.45$; $df = 6,58$; $P = 0.8438$),

leaf area ratio ($F = 0.13$; $df = 1,58$; $P = 0.7195$) and log height ratio ($F = 1.06$; $df = 1,58$; $P = 0.3067$) were significant. The interaction of generation*treatment ($F = 0.32$; $df = 2,58$; $P = 0.7260$) was also non-significant. Beetle preference was found to be unaffected by cultivar (Figure 3).

Experiment 3

Analysis revealed mildly significant effects of day ($F = 2.06$; $df = 5,45$; $P = 0.0880$) and height ratio ($F = 2.98$; $df = 1,45$; $P = 0.0912$). The effects of generation ($F = 0.07$; $df = 1,45$; $P = 0.7905$), treatment ($F = 1.87$; $df = 2,45$; $P = 0.1656$), defoliation ratio ($F = 0.18$; $df = 1,45$; $P = 0.6749$), leaf area ratio ($F = 0.65$; $df = 1,45$; $P = 0.4238$) and the interaction of generation*treatment ($F = 0.75$; $df = 2,45$; $P = 0.4775$) were found to be non-significant. Least squares mean analysis revealed mildly significant ($P < 0.10$) preference for the 7% LA ($t = -1.71$; $df = 45$; $P = 0.0937$) and 3% LA ($t = -1.93$; $df = 45$; $P = 0.0598$) cultivars compared to the 1% LA cultivar (Figure 3). Compared to the 1% LA cultivar, BLBs were 2.69 times more likely to choose the 7% LA cultivar and 4.12 times more likely to select the 3% LA cultivar.

Discussion

We observed a BLB preference for the induced volatiles compared to constitutive volatiles of the 7% LA cultivar (Figure 2). This result was in agreement with O'Neill et al. (2010), which found a similar preference for induced soybean volatiles using Japanese beetles. It is also in agreement with

other studies that have found soybean to be capable of inducing a chemical response upon BLB feeding (Felton et al. 1994, Srinivas and Danielson 2001, Srinivas et al. 2001a, 2001b). In comparison to the previous studies, which found BLB damage to deter further BLB feeding, we measured preference for plant volatiles as opposed to feeding preference (Felton et al. 1994, Srinivas and Danielson 2001, Srinivas et al. 2001a, 2001b). In this manner we measured plant volatile response to feeding as opposed to direct plant defenses.

The preference we observed for 7% LA induced volatiles in Experiment 1, was not observed for the 1% LA cultivar. This led to our hypotheses about the differences in volatile blends between commodity soybean cultivars (7% LA) and altered linolenic acid cultivars (1% LA and 3% LA). Bean leaf beetle preference for the volatiles of unwounded plants was not influenced by the cultivars used in our experiments. Results for Experiment 2 testing differences in constitutive volatiles emissions did not show any difference among cultivars (Figure 3). This result led us to reject our first possible hypothesis that altered linolenic acid cultivars constitutively produced volatile blends similar to those induced by BLB feeding on commodity cultivars.

The results of Experiment 3 suggest that the altered fatty acid synthesis pathway of the 1% LA cultivar resulted in changes to the induced volatile profile of the soybean plant, making the plant less attractive to BLBs (Figure 3). The results also suggest that other plant characteristics such as plant height, leaf area and leaf area removed are important in determining a beetle's preference for a

soybean plant. O'Neill et al. (2010) found that both prior feeding by Japanese beetles and growing plants in a high CO₂ environment increased the attractiveness of soybean plants for Japanese beetles. In both cases, prior herbivory and high CO₂, the authors found an elevation in the production of green leaf volatiles. Agelopoulos et al. (2000) found in intact potato, plant weight and plant age are characteristics that significantly impact total volatile emission. Our variables of plant height and leaf area may correlate to either of these characteristics. Also our variable of leaf area removed approximates the extent of damage inflicted on wounded plants, which can also have quantitative impacts on volatile emissions (Copolovici et al. 2011).

The results of Experiment 1 show female bean leaf beetles are attracted to the induced volatile profiles of our 7% LA commodity cultivar. Other chrysomelid herbivores are also attracted to induced plant volatiles including the crucifer flea beetle, *Phyllotreta cruciferae* Goeze, the saltcedar biocontrol agent *Diorhabda elongata* Brulle and the Colorado potato beetle (Bolter et al. 1997, Landolt et al. 1999, Cosse et al. 2006). Linolenic acid derived green leaf volatiles are important components in inducible potato plant response to Colorado potato beetle (WeiBbecker et al. 1997 and Gosset et al. 2008) and beetle attraction to damaged potato plants (Schutz et al. 1995).

Colorado potato beetles and BLB life histories show many parallels that may explain similar host plant attraction cues. Colorado potato beetles also overwinter as adults primarily in field edges such as hedgerows and wooded lots

(Milner et al. 1992). They also feed primarily on an agricultural crop that is grown in rotation. This necessitates the use of an early season search for new host plant fields. The flight capacity of both BLBs (Krell et al. 2003) and Colorado potato beetles (Weber et al. 1993) have been characterized using flight mills and found to be similar. Colorado potato beetles and bean leaf beetles use many short trivial flights (Colorado potato beetle ≤ 10 m; bean leaf beetle ≤ 30 m) to move within and between fields. Both species of beetles have similar slightly aggregated within field distributions (Kogan et al. 1974, Boiteau et al. 1979, Smelser and Pedigo 1992b, Taylor 1961). The use of multiple short flights for movement by both beetles may facilitate the use of plant volatiles for location of fields of host plants. The attraction of both species to damaged plants may be one mechanism to explain the slightly aggregated in-field dispersions of both beetles.

Our experiments did not utilize genetic isolines of soybean. We set out to analyze the impact of altered fatty acid synthesis pathways for the purpose of lowering seed linolenic acid levels on soybean plant volatile emissions. There are likely large genetic background differences among the three cultivars we used. This prevents us from definitively determining the reason for the differences in BLB responses in Experiments 1 and 3. Srinivas et al. (2001b) tested the induced resistance response against BLB of three soybean entries adapted to the north central United States. They found no significant differences in induced resistance response among the three entries. Felton et al. (1994)

found soybean capable of inducing resistance to *Helicoverpa zea* feeding after prior feeding by BLB. They also found BLB feeding to up-regulate the activity of lipoxygenase activity. The previous findings of Srinivas et al. (2001b), suggesting little variation among cultivars in inducible defenses, and Felton et al. (1994), implicating linolenic acid derived defense pathways, suggest that the differences in fatty acid synthesis pathways between our cultivars may be responsible for the differences in BLB preference we observed. The results of Experiments 1 and 3 therefore implicate the potential importance of linolenic acid in the soybean plant's induced volatile response to BLB feeding.

The results of our experiments demonstrate that female BLBs are preferentially attracted to the volatiles of wounded soybean plants when compared to unwounded plants. This attraction may involve linolenic acid derived volatile compounds as suggested by a preference for the induced volatiles of the 7% LA and 3% LA cultivars compared to the 1% LA cultivar. Also a lack of preference for wounded plants as compared to unwounded plants of the ultra-low-linolenic acid cultivar IA 3028 supports this conclusion. Other factors including other induced plant volatiles, plant characteristics such as plant age, leaf area or amount of defoliation may be involved in determining BLB attraction for the induced volatiles of a soybean plant.

Results from our experiments were mildly significant ($0.05 < P < 0.10$), but we believe them to be robust given the large number of BLBs (1,880) from which our conclusions are derived. Our experiments suggest that linolenic acid derived

induced soybean volatiles can be unintentionally modified by plant breeding and that these modifications can have an impact on host plant selection of herbivorous insects. Future research is needed to understand the specific volatile chemicals that are modified, the impact of this modification on host plant location and selection of different herbivores and the implications for soybean management.

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Figure Captions

Figure 1. The olfactometer apparatus that was used to measure bean leaf beetle preference for different soybean volatile profiles is depicted. Plants with varying levels of seed linolenic acid content and wounding by bean leaf beetles were placed in the two satellite chambers. Plants were covered with fine mesh nets to prevent beetles from feeding on the plants during the experiment. Ten female bean leaf beetles were placed into the central chamber at the start of each experiment. The beetles' movement was monitored for 24 hours and their location in the olfactometer was recorded at 24 hours after the start of the experiment.

Figure 2. The average number of beetles selecting one soybean plant over a second in Experiment 3 is depicted. Beetle selection was based on the volatile profiles of an unwounded and wounded soybean plant. Plant wounding was accomplished by placing two female bean leaf beetles on a plant 24 hours prior to the start of an experiment. The two beetles were removed just prior to the start of the experiment. Odds ratios were calculated to assess beetle preference for the volatile profiles of a wounded or unwounded plant. Asterisks (*) depict significant beetle preference for one cultivar over another at the $P = 0.10$ level.

Figure 3. The average number of beetles selecting one soybean cultivar over a second is depicted. Beetle selection was based on the volatile profiles of unwounded (Experiment 2) and wounded (Experiment 3) soybean plants varying in seed linolenic acid content. Beetle preference for a given cultivar

compared to a second was analyzed using odds ratios. Asterisks (*) depict significant beetle preference for one cultivar over another at the $P = 0.10$ level.

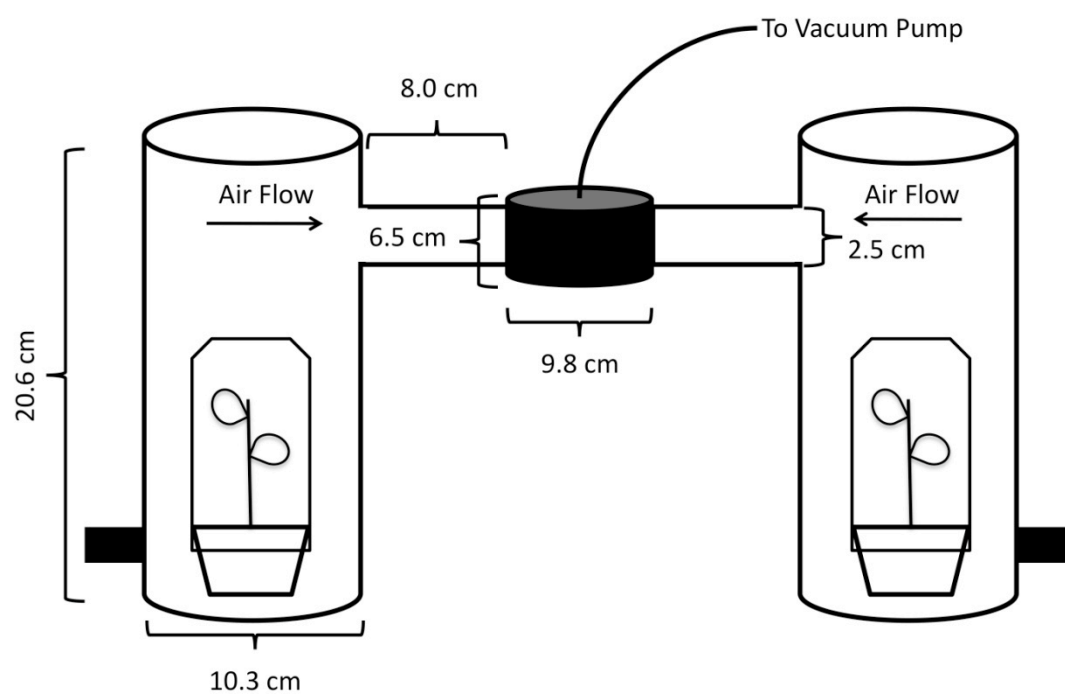
Figure 1.

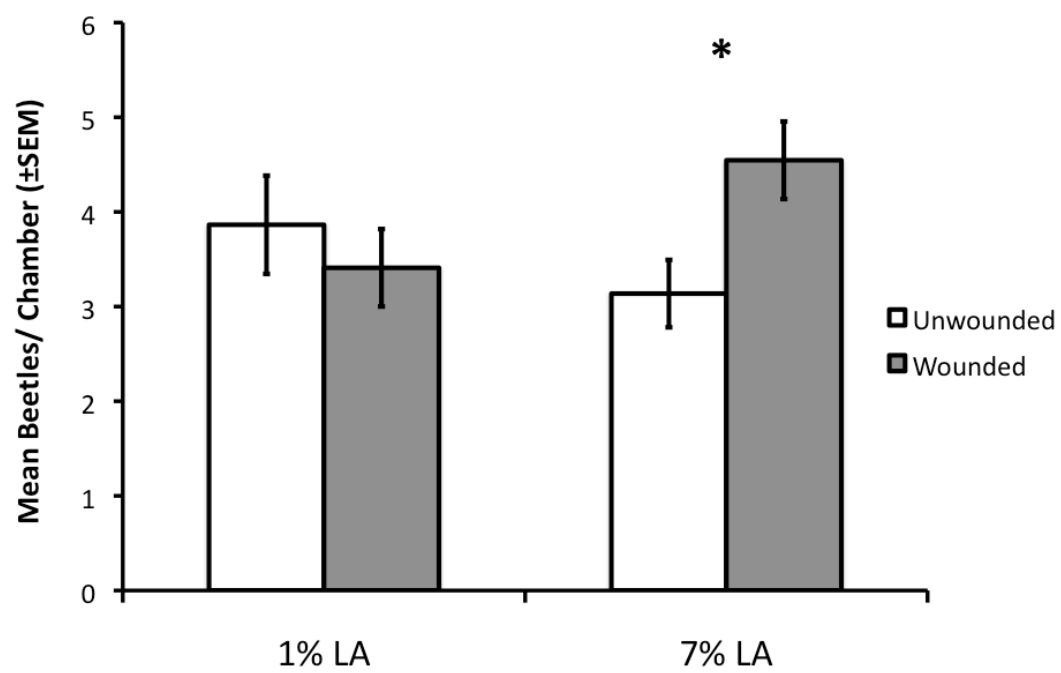
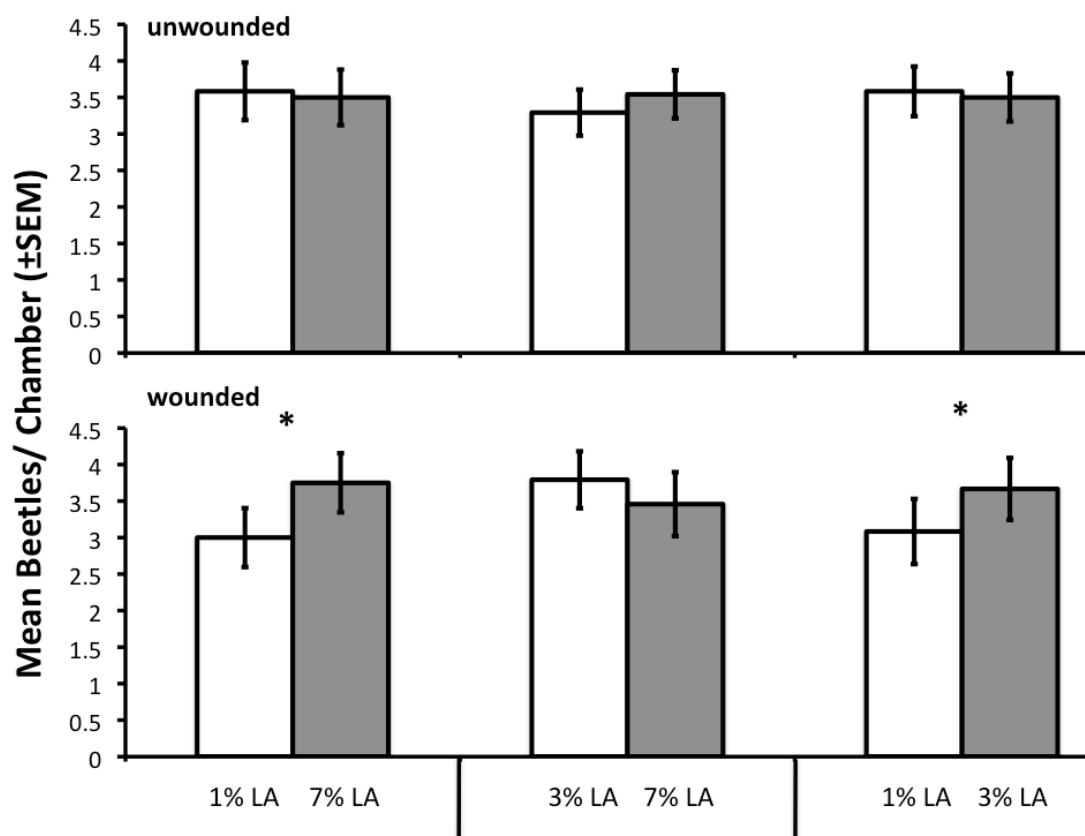
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Figure 3.



Chapter 6

General Conclusions

The goal of this research was to further our knowledge of soybean integrated pest management (IPM) tactics. Our goal was to evaluate recommended IPM tactics and their effectiveness for a changing agricultural environment. We sought to investigate the impacts of a reduction in insect derived ecosystem services, the potential for interactions between introduced soybean pests and a shift in the soybean cultivars being grown.

Integrated pest management tactics were developed using conventional soybean cultivars and environmental conditions current to the time. Since the invasion of *Aphis glycines*, pesticide application in soybean has risen drastically (NASS 1999, 2005). Concurrent with the rise in pesticide application was an increase in corn acreage due to an increasing demand for biofuels (Landis et al. 2008). These two factors have been implicated in a reduction in the ecosystem services provided by insects in soybean. The results of chapter two indicate that the current economic threshold for *A. glycines* (Ragsdale et al. 2007) will still provide efficient yield protection even with a complete removal of bio-control services provided by insects. This result is most likely due to an underestimation of the importance of abiotic factors in slowing *A. glycines* population growth at densities between the economic threshold and economic injury level.

Aphis glycines is a recently invasive pest to North America (Ragsdale et al. 2004). It has been proposed that the soybean aphid is part of an invasional

meltdown (Simberloff and Von Holle 1999) that includes soybean, *Rhamnus cathartica*, *Harmonia axyridis* and *Coccinella septempunctata* (Ragsdale et al. 2011). An invasional meltdown occurs when the establishment of one invasive species makes it easier for another to establish. The results of chapter three indicate that as part of the soybean-*R. cathartica*-*A. glycines*-*H. axyridis* and *C. septempunctata* invasional meltdown the soybean cyst nematode, *Heterodera glycines* which has already benefited from the establishment of soybean (Niblack et al. 2006) may further benefit from the establishment of *A. glycines*. Chapter three also indicates that the positive effects of *A. glycines* on *H. glycines* could have large impacts on *H. glycines* management recommendations. The results of this chapter will have to be further investigated in order to develop management techniques that integrate *A. glycines* and *H. glycines* management. An early result from chapter three indicates that these management techniques may include the use of *A. glycines* resistance stacked with PI 88788 derived *H. glycines* resistance, which also displayed activity against *A. glycines*.

Over the last three decades specialty soybean cultivars have been developed with altered grain traits (Fehr 2006). Current integrated pest management tactics were developed for conventional soybean cultivars with a primary focus on yield and grain quality (Adee et al. 1994, Niblack 2005, Ragsdale et al. 2007). How these management tactics apply for altered fatty acid soybean, where grain composition as well as yield and grain quality are important, was unknown. The results of chapter four indicate that soybean pests are capable of impacting both the yield and

seed linolenic acid content of low linolenic acid soybean cultivars. However, chapter four validates current management recommendations for low linolenic acid soybean production.

Furthering our knowledge of how specialty low linolenic acid soybean cultivars may affect management, chapter five investigated the impact of these cultivars on *Cerotoma trifurcata*, olfactory preference. The results of our experiments indicate that *C. trifurcata* are attracted to volatiles emitted by wounded conventional soybean cultivars. The mutations in fatty acid synthesis pathways in the ultra-low linolenic acid cultivar led to a decreased preference for wounded plants of the cultivar. This experiment indicates that for ultra low linolenic acid cultivars the reduction in seed linolenic acid levels had an impact on induced plant volatiles and this difference was capable of being perceived by an insect pest. How this change in volatile profile may affect host-finding behavior of other soybean insect pests is unknown.

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